UNIVERSITY OF ALBERTA

CHARACTERIZATION OF RHEOLOGICAL AND FUNCTIONAL PROPERTIES OF BARLEY β-GLUCAN EXTRACTED AT LABORATORY AND PILOT PLANT

BY

ZVONKO BURKUS ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of

the requirements for the degree of **DOCTOR OF PHILOSOPHY**

IN

FOOD SCIENCE AND TECHNOLOGY

DEPARTMENT OF AGRICULTURAL, FOOD AND NUTRITIONAL SCIENCE

EDMONTON, ALBERTA

FALL, 2001

National Library
of Canada

Acquisitions and
Bibliographic Services

395 Wellington Street
Ottawa ON K1A 0N4
Canada

Bibliothèque nationale
du Canada

Acquisitions et
services bibliographiques

395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file Votre référence

Our file Notre référence

The author has granted a non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of this thesis in microform, paper or electronic formats.

The author retains ownership of the copyright in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

L'auteur a accordé une licence non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de cette thèse sous la forme de microfiche/film, de reproduction sur papier ou sur format électronique.

L'auteur conserve la propriété du droit d'auteur qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

0-612-68915-8

Canadä

University of Alberta

Library Release Form

Name of Author: Zvonko Burkus

Title of Thesis: CHARACTERIZATION OF RHEOLOGICAL AND FUNCTIONAL PROPERTIES OF BARLEY β-GLUCAN EXTRACTED AT LABORATORY AND PILOT PLANT

Degree: Doctor of Philosophy

Year this Degree Granted: 2001

Permission is hereby granted to the University of Alberta Library to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves all other publication and other rights in association with the copyright of thesis, and except as herein before provided, neither the thesis nor any substantial portion thereof may be printed or otherwise reproduced in any material form whatever without the author's prior written permission.

# 8 11325-103 Av.
T5K 0S3
Edmonton, Alberta, Canada

Date: August 31st, 2001.

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that hey have read, and recommended to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled CHARACTERIZATION OF RHEOLOGICAL AND FUNCTIONAL PROPERTIES OF BARLEY β-GLUCAN EXTRACTED AT LABORATORY AND PILOT PLANT submitted by Zvonko Burkus in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Food Science and Technology.

F. Temelli

Dr. F. Temelli (Supervisor)

Peter Sporns

Dr. P. Sporns (Committee member)

Dr. T. Vasanthan (Committee member)

R T Tyler

Dr. R. T. Tyler

Dr. S. R. Jeffrey

Date: Aug. 31/2001

ABSTRACT

β-Glucan is a hydrocolloid abundant in oats and barley. It has blood glucose regulating, hypocholesterolemic and immunostimulating effects while exhibiting thickening, food stabilizing and fat replacing potential.

Barley β-glucan (BBG) gum was extracted at pilot plant (PP) and laboratory (LAB) scale at pH 9 and 55°C, the starch- and protein-rich phases separated and the extract stabilized at 90°C for 1 h. Molecular weights of PP and LAB gums were determined using intrinsic viscosity measurements. Both gums were tested for pseudoplasticity, thixotropy, temperature dependence of viscosity, yield point and network development at different concentrations over time. Their stabilities at high shear stress, under freeze-thawing conditions and at different pH-temperature-time combinations were investigated, and their emulsion and foam forming and stabilizing potentials assessed.

Pilot Plant and LAB BBG extractions resulted in gums with 3.5% and 5.5% yields, 83.3% and 78.0% purities and viscosities of 18 and 668 mPa s (1% w/w, measured at 12.9 1/s), respectively. Lower viscosity of PP gum was mostly due to high shear during separation of phases and less to the extended holding at high pH and elevated temperature. Apparent molecular weights were determined to be 198K for PP and 598K for LAB gum. Determination of critical concentration as $c^* \approx 2.5/[\eta]$ was suitable for high viscosity (LAB) BBG gums but not for low viscosity (PP) gums.

Fresh solutions of both BBG gums were not thixotropic at ≤1% (w/w) concentrations. Viscosities of both gums decreased with temperature, as expected. Flow behavior index at low shear rates was ≥0.8 for LAB gum above 15°C and >0.99 for PP gum at 0.1-75°C. Network formation for low viscosity gums was rapid at 5% concentration when elastic modulus G' exceeded viscous modulus G'' after ≥1.5 h with measurable yield point.

High shear was detrimental for LAB gum, whereas PP gum was resistant to homogenization, probably as the consequence of different chain length and micellar structure. Laboratory gum solution was more resistant to freeze-thawing, but less resistant to extreme pH, especially at higher temperatures. Low viscosity PP gum underwent reversible network formation over time regardless of environmental pH. Foam and emulsion stabilization were most efficient when LAB gum was combined with whey protein concentrate.

High viscosity LAB gum shows potential for application in food systems, whereas the behavior of PP gum represents a challenge for future research.

TABLE OF CONTENTS

Title **Page**

1. INTRODUCTION AND LITERATURE REVIEW 1
- 1.1. INTRODUCTION AND OBJECTIVES OF THESIS 1
- 1.2. LITERATURE REVIEW 3
  - 1.2.1. BARLEY PRODUCTION 3
  - 1.2.2. NEW BARLEY VARIETIES 6
  - 1.2.3. β-GLUCAN PROPERTIES 7
    - 1.2.3.1. Health promoting properties 7
    - 1.2.3.2. Spatial configuration of β-glucan 8
    - 1.2.3.3. Thermodynamic incompatibility of β-glucan 10
  - 1.2.4. EXTRACTION OF β-GLUCAN 12
    - 1.2.4.1. Yield of BBG gum 13
    - 1.2.4.2. Purity of BBG gum 14
    - 1.2.4.3. Viscosity of BBG gum 17
    - 1.2.4.4. Stability of BBG gum 18
    - 1.2.4.5. Rheological properties of β-glucan 21
    - 1.2.4.6. Applications of β-glucan 25
- 1.4. REFERENCES 27

2. PILOT PLANT AND LABORATORY SCALE EXTRACTION OF BARLEY β-GLUCAN GUM 33
- 2.1. INTRODUCTION 33
- 2.2. MATERIALS AND METHODS 37
  - 2.2.1. Materials 37
  - 2.2.2. Extraction of β-glucan 37
  - 2.2.3. Chemical analyses 38
  - 2.2.4. Viscosity measurements 40
- 2.3. RESULTS AND DISCUSSION 41
  - 2.3.1. Yield, composition and viscosity of gums 41
  - 2.3.2. Stability of BBG gum 49
- 2.4. CONCLUSIONS 51
- 2.5. REFERENCES 52

3. DETERMINATION OF THE MOLECULAR WEIGHT OF BBG USING INTRINSIC VISCOSITY MEASUREMENTS 54
- 3.1. INTRODUCTION 54
- 3.2. MATERIALS AND METHODS 56
  - 3.2.1. Materials 56
  - 3.2.2. Preparation of β-glucan solutions 56
  - 3.2.3. Viscosity measurements 57
  - 3.2.4. Determination of intrinsic viscosity, molecular weight and critical concentration 58

Title **Page**

3.3. RESULTS AND DISCUSSION 59
  3.3.1. Intrinsic viscosity and molecular weight 59
  3.3.2. Critical concentration 65
3.4. CONCLUSIONS 67
3.5. REFERENCES 68

4. RHEOLOGICAL PROPERTIES OF BARLEY β-GLUCAN 70
4.1. INTRODUCTION 70
4.2. MATERIALS AND METHODS 73
  4.2.1. Materials 73
  4.2.2. Rheological measurements 73
4.3. RESULTS AND DISCUSSION 76
  4.3.1. Effect of temperature and shear on viscosity 76
  4.3.2. Thixotropy 83
  4.3.3. Network formation 86
  4.3.4. Yield point 94
4.4. CONCLUSIONS 99
4.5. REFERENCES 101

5. INFLUENCE OF PREPARATION, SHEAR AND STORAGE TREATMENTS ON BARLEY BBG SOLUTIONS 103
5.1. INTRODUCTION 103
5.2. MATERIALS AND METHODS 105
  5.2.1. Materials 105
  5.2.2. Treatment methods 105
  5.2.3. Rheological measurements 108
  5.2.4. Scanning electron microscopy 109
  5.2.5. Statistical analysis 109
5.3. RESULTS AND DISCUSSION 109
  5.3.1. Solution preparation treatments 109
  5.3.2. Shear effect 110
  5.3.3. Freeze-thawing of BBG solutions 118
  5.3.4. Effect of long term refrigerated storage 122
  5.3.5. Scanning electron microscopy 128
5.4. CONCLUSIONS 135
5.5. REFERENCES 137

6. THE INFLUENCE OF pH AND TEMPERATURE ON THE VISCOSITY OF BARLEY β-GLUCAN 139
6.1. INTRODUCTION 139

Title **Page**

6.2. MATERIALS AND METHODS.......... 141
  6.2.1. Materials.......... 141
  6.2.2. High temperature tests at pH 2-10.......... 141
  6.2.3. Long term storage tests.......... 142
  6.2.4. Viscosity measurements.......... 143
  6.2.5. Statistical analysis.......... 143
6.3. RESULTS AND DISCUSSION.......... 143
  6.3.1. High temperature tests at pH 2-10.......... 143
  6.3.2. Long term storage tests.......... 150
6.4. CONCLUSIONS.......... 154
6.5. REFERENCES.......... 156

7. STABILIZATION OF EMULSIONS AND FOAMS USING BARLEY β-GLUCAN.......... 158
7.1. INTRODUCTION.......... 158
7.2. MATERIALS AND METHODS.......... 161
  7.2.1. Materials.......... 161
  7.2.2. Sample preparation.......... 161
  7.2.3. Physical properties determination.......... 162
  7.2.4. Stability of foams and emulsions.......... 164
  7.2.5. Statistical analysis.......... 165
7.3. RESULTS AND DISCUSSION.......... 165
  7.3.1. Physical properties.......... 165
  7.3.2. Stability of foams and emulsions.......... 172
7.4. CONCLUSIONS.......... 178
7.5. REFERENCES.......... 179

8. CONCLUSIONS AND RECOMMENDATIONS.......... 182
8.1. REFERENCES.......... 193

LIST OF TABLES

Table **Page**

1.1. World production of barley, 1991-2000 (FAO 2000)............................... 4

1.2. Production of barley in Canada, 1991-2000 (FAO 2000).......................... 5

2.1. Mass balance of fractions from 200 kg of barley flour (89.3 % solids) in pilot plant extraction and 200 g (95.9% solids) in laboratory extraction of BBG gums.. 42

2.2. Composition of fractions obtained in PP and LAB scale extraction of β-glucan gum.. 43

2.3. Coefficients of pseudoplasticity of 1% (w/w) PP and LAB gum solutions according to Power Law equation.. 45

3.1. Intrinsic viscosity [η] of MW standards, PP and LAB gums after linear extrapolation from ≤0.1% concentration to c→0, or polynomial extrapolation from 0.2% to c→0.. 62

4.1. Parameters in the measurement window for the measurement of PP and LAB gum thixotropy at 1000 - 3000 rpm and 20°C................................ 74

4.2. Power Law constants for 1% (w/w) LAB gum viscosity curves in the range 1-20 rpm (1.29-26.9 s^{-1}) at 0.1-75°C.. 79

4.3. Power Law constants for 1% (w/w) PP gum viscosity curves in the range 1-20 rpm (1.29-26.9 s^{-1}) at 0.1-75°C.. 80

4.4. Activation energy equations and coefficients calculated from Arrhenius plot for 1% PP and LAB gums at 0.1-20°C 85

4.5. Yield point data for PP and Condor gels after overnight setting. Amplitude sweep was 2-100% strain at 1 Hz frequency.................................... 98

5.1. Changes in the apparent viscosity of 1% (w/w) PP and LAB gum solutions after 4 Freeze-Thaw (F-T) cycles as measured at 10 rpm (12.9 s^{-1}) and 20°C.. 120

5.2. Changes in the apparent viscosity of the top layer of 1% (w/w) PP and LAB solutions after one ("accidental") Freeze-Thaw (F-T) cycle and subsequent refrigerated storage. Viscosity measured at 10 rpm (12.9 s^{-1}) and20 °C........ 124

Table **Page**

5.3. Changes in the apparent viscosity of 1% (w/w) PP disturbed and not disturbed solutions during storage and 1% (w/w) LAB gum solutions over 4-week storage at 4°C. Viscosity measured at 10 rpm (12.9 s^{-1}) and 20°C................ 125

5.4. Power Law constants for 1% PP gum solution over 4 week storage at 4°C. Viscosity curves measured at 20°C in the range of 1-20 rpm (1.29-26.9 s^{-1}) for PP gum solutions and 1-100 rpm (1.29-129 s^{-1}) for LAB gum solutions.... 127

6.1. Changes in the apparent viscosity of 1% (w/w) PP gum solutions at 10 rpm and pH 2-10 after 1 h thermal treatment at 55 and 90°C......................... 144

6.2. Changes in the apparent viscosity of 0.5% (w/w) LAB gum solutions at 10 rpm and pH 2-10 after 1h thermal treatment at 55 and 90°C.................. 145

6.3. Changes in the apparent viscosity of 1% PP and 0.5% LAB solutions measured at 10 rpm (12.9 s^{-1}) and 20°C during 4-week storage at room temperature and different pH 151

6.4. Power Law flow behavior index (n) for 1% PP and 0.5% LAB gum solutions at different pH during 4 week storage at room temperature. Viscosity curves measured in the range 1-20 rpm (1.29-26.9 s^{-1}) at 20°C....... 152

7.1. Droplet size and creaming of emulsions prepared with 1% (w/w) WPC and 1% (w/w) BBG gum prepared in the laboratory (LAB) or pilot plant (PP).... 173

LIST OF FIGURES

Figure **Page**

1.1. Possible structure of β-glucan micelles 11

1.2. Frequency dependence (ω) of storage modulus G', loss modulus G'' and dynamic viscosity η* for polysaccharide systems 24

2.1. Flow chart of laboratory and pilot plant extraction of barley β-glucan from whole barley flour 39

2.2. Effect of shear rate on viscosity of LAB and PP gums at 1% (w/w) concentration 45

2.3. Viscosity stability of 1% (w/w) PP gum solution and fresh LAB extract as a function of time 50

3.1. Viscosity of MW standards, PP and LAB gums in the concentration range 0.25-1% (w/w) 60

3.2. Reduced viscosities of BBG MW standards, PP and LAB gum in the concentration range 0.025-0.2% (w/w) 61

3.3. Intrinsic viscosity standard curve 63

4.1. Viscosity of 1% (w/w) LAB gum solution at 0.1-20°C (A), 25-75°C (B) determined at 1-20 rpm 77

4.2. Viscosity of 1% (w/w) PP gum solution at 0.1-20°C (A), 25-75°C (B), determined at 1-20 rpm 78

4.3. Concentration dependence of viscosity vs. shear rate for (A) LAB gum, and (B) Condor (open symbols) and PP gum solutions (solid symbols) 82

4.4. Arrhenius plot for viscosity of 1% (w/w) LAB gum solution over the temperature range 0.1-75°C and at four shear rates (1.29-25.8 s^{-1}) 84

4.5. Thixotropy of 1% (w/w) LAB gum solution after shearing at 1000 and 3000 rpm at 20°C 87

4.6. Thixotropy of 1% (w/w) PP gum solution after shearing at 1000 rpm and 20°C 88

Figure **Page**

4.7. Thixotropy of 1% PP gum and 0.25% LAB gum solutions after shearing at 1000 and 3000 rpm and 20°C 89

4.8. Storage modulus (G') and loss modulus (G'') development vs. time for a 5% (w/w) solution of Condor gum determined at 5% strain and 1 Hz frequency 90

4.9. Storage modulus (G') and loss modulus (G'') development vs. time for 2.5% (w/w) solution of Condor gum determined at 5% strain and 1 Hz frequency 92

4.10. Storage modulus (G') and loss modulus (G'') development in time for 5% (w/w) PP gum solution determined at 3% strain and 0.5 Hz frequency 93

4.11. Amplitude sweep at 2-100% strain and 1 Hz frequency for 5% (w/w) Condor gum before and after overnight test 95

4.12. Amplitude sweep at 2-100% strain and 1 Hz frequency for 2.5% (w/w) Condor gum after overnight test 96

4.13. Amplitude sweep at 2-100% strain and 1 Hz frequency for 5% (w/w) PP gum after overnight test 97

5.1. The effect of Polytron shear (Poly) and ultrasonication (U-sonic) on the viscosity of 0.7% (w/w) LAB gum after 60 s treatment 111

5.2. The effect of Polytron shear (Poly) and ultrasonication (U-sonic) on the viscosity of 0.5% (w/w) LAB gum after 60 s treatment 112

5.3. The effect of Polytron shear (Poly) and ultrasonication (U-sonic) on the viscosity of 0.25% (w/w) LAB gum after 60 s treatment 113

5.4. The effect of Polytron shear (Poly) and ultrasonication (U-sonic) on the viscosity of 1% (w/w) PP gum after 60 s treatment 114

5.5. The effect of Polytron shear (Poly) and ultrasonication (U-sonic) on the viscosity of 0.5% (w/w) PP gum after 60 s treatment 115

5.6. Viscosity change in top and bottom layers of 0.5% (w/w) LAB gum after 4 freeze-thaw (F-T) cycles 121

Figure **Page**

5.7. Viscosity change in top and bottom layers of 0.25% (w/w) LAB gum after 4 freeze-thaw (F-T) cycles.......... 123

5.8. Scanning electron micrograph of 1% (w/w) fresh LAB solution (x1000)........ 129

5.9. Scanning electron micrograph of 1% (w/w) fresh PP solution (x1000).......... 130

5.10. Scanning electron micrograph of 0.5% (w/w) fresh LAB solution (x1000).... 131

5.11. Scanning electron micrograph of 1% (w/w) PP solution after one F-T cycle (x1000).......... 132

5.12. Scanning electron micrograph of 3% (w/w) PP solution after setting a network in a refrigerator for a week (x1500).......... 133

5.13. Scanning electron micrograph of 0.5% (w/w) fresh PP solution under higher magnification (x100,000).......... 134

6.1. Viscosity change in 0.5% (w/w) LAB, 0.5% (w/w) Pure BBG and 1% (w/w) PP solutions after 4 x 1 h consecutive heat treatments at 90°C.......... 148

6.2. Viscosity change of 1% (w/w) PP prepared with pH 4 buffer during 4 week storage at room temperature.......... 153

7.1. Surface tension of BBG gum and WPC solutions measured as a function of time.......... 162

7.2. Interfacial tension of BBG gum and WPC solutions measured as a function of time.......... 168

7.3. Differential scanning calorimetry at 5°C/min for different barley β-glucan gums at approximately 5% (w/w) concentration.......... 169

7.4. Enlarged DSC of approximately 5% (w/w) LV-pure BBG gum at 5°C/min..... 170

7.5. Volume of foams prepared with 5% WPC, 1% PP or LAB gum and 10% sugar.......... 175

7.6. Time to 50% drainage of foams prepared with 5% WPC, 1% PP or LAB BBG gum and 10% sugar.......... 176

LIST OF ABBREVIATIONS

AACC	American Association of Cereal Chemists
ADSA-P	axisymmetric drop shape analysis-profile
AGC	Automatic Gap Control
BBG	barley β-glucan
CSD	controlled shear displacement
DSC	differential scanning calorimetry
DW	distilled water
EtOH	ethanol
FDA	Food and Drug Administration (in US)
F-T	freeze-thawing
GOPOD	glucose oxidase/peroxidase
HF	heavy fraction
HV	high viscosity
K	1000
LAB	laboratory
LSD	least significant difference
LV	low viscosity
MV	medium viscosity
MW	molecular weight
OBG	oat β-glucan
PP	pilot plant

Poly	Polytron
SEM	scanning electron microscopy
TDF	total dietary fiber
U-sonic	ultrasonication
WPC	whey protein concentrate
WPI	whey protein isolate

FOREWORD

Researchers, especially young ones, are prone to repeat experiments and conclusions without ever questioning them, even more so if they are coming from top names in our "business". We forget, including myself in the beginning, that not all details are ever disclosed in research papers although we claim to do that when we write them. Every conclusion is strongly limited to the conditions and variables of a performed experiment. Furthermore, there is no guarantee that conclusions are always true and universally applicable. Suspicion is a scientist's best friend and the best we can achieve is that we are only partially correct. It is just a matter of time before somebody will prove that our thoughts were, at best, partially wrong, incomplete or narrowly applicable. Simply, we don't know enough. That's why we are in this business; to search for the truth.

1. INTRODUCTION AND LITERATURE REVIEW

1.1. INTRODUCTION AND OBJECTIVES OF THESIS

Barley is probably the oldest cereal used for human consumption. Throughout history it was the staple food in many countries. However, due to industrialization and preference for wheat products by modern humans, consumption of barley as a food has steadily declined, reaching the level of 5-10% of total production in western countries. Recent discoveries of health stimulating properties of barley (and oat) β-glucan, such as hypocholesterolemic, regulation of blood glucose, immunostimulation and cancer fighting properties, have renewed interest in barley. Advances in breeding with the creation of new hulless varieties with waxy starch, which are even richer in more viscous β-glucan, stimulated research, resulting in a few patents related to the extraction of barley and oat β-glucan.

A further boost to research resulted from the U.S. Food and Drug Administration (FDA) approval of a health claim in 1997 for products rich in oat β-glucan indicating that "soluble fiber from oatmeal, as part of a low saturated fat, low cholesterol diet may reduce the risk of heart disease", but the same claim was not extended to oat β-glucan gum. Both oat and barley β-glucan gums, if extracted as high-viscosity (HV) gums, are comparable to widely used guar and locust bean gums in terms of their rheological properties. However, our knowledge about β-glucan gums, such as their long term stability after extraction, gelling properties and resistance to cleavage at different conditions of industrial processing, is incomplete. Therefore, a better understanding of the behavior of β-glucan under different conditions and in food systems is essential for enhanced commercial application of β-glucan gums.

Recently, in our laboratory, a major effort was undertaken to incorporate barley β-glucan (BBG) extracted at pilot plant scale into an orange-flavored beverage as a source of fiber and stabilizer (Bansema 2000) and into breakfast sausages as a fat replacer (Morin 2001), with encouraging results. In order to make such applications of BBG commercially successful, further research should be undertaken to enrich our knowledge about the physical stability during processing and shelf stability of β-glucan under various conditions. Therefore, the objectives of this thesis were:

- to extract stable, high purity, HV BBG at the pilot plant (PP) and laboratory (LAB) scale. The procedure should eliminate ethanol reflux as a means of stabilization, recover high yields of BBG and gain an insight into further improvement of the extraction process.
- to determine the molecular weight of PP and LAB BBG using intrinsic viscosity measurements.
- to verify rheological properties of PP and LAB BBG gums. Properties such as viscosity at different temperatures, pseudoplasticity, thixotropy, yield point and viscoelastic behavior at different concentrations should be determined.
- to study BBG stability under different conditions such as high shear stress, freeze-thawing and pH-temperature-time combinations.
- to investigate emulsion and foam forming and stabilizing properties of PP and LAB BBG gums.

Obtained results should provide a valuable insight into physical properties and functionality of β-glucan and establish a basis for further research, with the ultimate goal of commercialization of barley as a food ingredient source.

1.2. LITERATURE REVIEW

Barley β-glucan, its occurrence, structure, health effects, extraction, functional properties and food applications were thoroughly reviewed by Burkus (1996). Therefore, in this review, more emphasis is placed on the latest research and relevant aspects that were not highlighted previously, as well as some observations and thoughts to help newcomers in the field.

1.2.1. BARLEY PRODUCTION

During the last decade, there has been a downward trend in world production of barley (Table 1.1). Production area has decreased, while yields show no change. The reason may be increased corn production, approximately 50% in the last twelve years (FAO 2000). Both commodities are destined primarily for the animal feed market and corn outperforms barley since it has higher yields, higher metabolizable energy and lower price in the world market. The quickly developing Asian market had a tendency to boost malting barley production through increased beer consumption, but the economic slowdown - "Asian flu" - delayed that trend.

Barley production for the year 2000 in Canada was 13.47 million tons - close to the 5 year average of 13.6 million tons (Statistics Canada 2000), with a slightly increasing trend for harvested area and yield (Table 1.2). Alberta production decreased in the year 2000, mostly due to drought in southeast Alberta when about 1 million acres of seeded

TABLE 1.1
World Production of Barley, 1991-2000 (FAO 2000).

	YEAR									
	1991	**1992**	**1993**	**1994**	**1995**	**1996**	**1997**	**1998**	**1999**	**2000**
Area, ha	76.3	73.5	74.3	72.7	69.1	66.0	64.2	61.3	56.4	55.7
Yield, t/ha	2.2	2.3	2.3	2.2	2.0	2.4	2.4	2.3	2.3	2.4
Production, 10^6 t	169.8	165.5	169.7	161.2	140.9	155.3	154.6	138.5	130.1	135.9

TABLE 1.2
Production of Barley in Canada, 1991-2000 (FAO 2000).

	YEAR									
	1991	**1992**	**1993**	**1994**	**1995**	**1996**	**1997**	**1998**	**1999**	**2000**
Area, ha	4.2	3.8	4.2	4.1	4.4	4.9	4.7	4.4	4.1	4.7
Yield, t/ha	2.8	2.9	3.1	2.9	3.0	3.2	2.9	2.9	3.2	3.1
Production, 10^6 t	11.6	11.0	13.0	11.7	13.0	15.6	13.5	12.7	13.2	14.6

area were not harvested. As a consequence, Saskatchewan exceeded Alberta for the first time in total production of barley with 5.5 million tons of barley harvested, mostly due to a 21% increase in seeded area (Statistics Canada 2000). Production of hulless barley gained significance and seeded area in Western Canada rose from about 86,500 ha in 1996 (Gosain 1996) to probably as much as 1 million ha in the year 2000 (Bhatty 2000). Hulless barley has higher metabolizable energy and protein content than its hulled predecessors and lower transportation and storage costs because of its higher hectoliter weight, which is comparable to that of wheat. Still, as a feed, it is cheaper than wheat and has higher yields in Canada, approximately 3.2 vs. approximately 2.6 t/ha (FAO 2000).

1.2.2. NEW BARLEY VARIETIES

Research efforts in this decade developed numerous new cultivars and lines of barley with special traits targeting new opportunities in food and non-food uses. In general, waxy varietes with low amylose content (≤5%) are richer in β-glucan, than their counterparts with regular starch. In addition, there is a new waxy barley, CDC Alamo, with zero amylose. Starch extracted from this variety has excellent freeze-thaw stability (Bhatty and Rossnagel 1997, Zheng et al 1998). There are also high amylose varieties with up to 45% amylose compared to an average of 25% amylose in regular varieties (Vasanthan and Bhatty 1995). Barley selection for high β-glucan established the cultivar Prowashonupana as a symbol for high fiber barley with 25.2% total dietary fiber (TDF) and up to 17.4% β-glucan (Bhatty 1999). Prowashonupana has about 35% starch and, consequently, low metabolizable energy, so that its use is limited to human consumption. These new cultivars are opening the door for wider barley value-added processing, especially in the food and paper industries.

1.2.3. β-GLUCAN PROPERTIES

1.2.3.1. Health promoting properties

The health promoting properties of β-glucan were reviewed by Newman and Newman (1991), Burkus (1996), Wood and Beer (1998) and Bhatty (1999). The major boost for β-glucan was the U.S. Food and Drug Administration's approval of the oat health claim (FDA 1997) for food products containing β-glucan from an oat source (bran, groats, rolled oats), which guarantees ≥0.75 g of β-glucan per serving. This claim was approved for oat products rather than for individual components. Oats also contain very potent antioxidants such as avenanthramides and oat saponins that, in minute amounts, have an hypocholesterolemic effect which equals that of β-glucan (Collins 1998). The activity of similar components in barley has not yet been reported. The oat health claim was filed by Quaker Oats (Chicago, IL), the company that dominates the oats market. Quaker Oats certainly benefited from the outcome in terms of increased sales even though they do not have exclusive rights to the claim. On the other hand, there is no major company to champion barley through such an extensive process and to bear the associated cost. However, efforts to obtain a similar claim for barley are underway. Needless to say, more clinical trials involving barley are needed to substantiate such a claim.

Not all of the clinical studies for the oat health claim have given positive results. An important parameter that has been overlooked is how the β-glucan source or the product containing that source was processed. Oats are routinely stabilized against lipolytic activity by steam treatment. This process also inactivates endogenous β-glucanases. For example, a cereal bar rich in oats could satisfy the requirements of the FDA claim. However, if the bar contained barley malt extract and was kept at increased moisture levels for some time, β-glucan would have been degraded. Beer et al (1997a) noticed that after freezing muffins enriched with oat, solubility of β-glucan was decreased, which probably corresponds to diminished health effects. Based on the above considerations, stabilization of the β-glucan source and optimization of handling and processing parameters are critical to maximizing the health effects of β-glucan.

Besides the widely elaborated cholesterol lowering and blood glucose regulating effects, there is new research to support the immunostimulating effect of β-glucan. Ceapro Inc. (Edmonton, AB) has been supplying oat β-glucan for the manufacture of special bandages impregnated with oat β-glucan (OBG) for burn and wound treatment. High viscosity (HV) and high molecular weight (MW) OBG had immunostimulating effect *in vitro* and *in vivo* in mice after intraperitoneal administration and increased the survival of mice infected with *Staphylococcus aureus* (Estrada et al 1997).

In vitro experiments of Causey et al (1998) showed that even hydrolyzed BBG had an immunostimulating effect on human macrophages. When applied at a concentration of 100 μg/mL, β-glucan increased production of white blood cells 6-fold. In addition, the anticancerogenic effect of barley bran (13% TDF) on the incidence and development of certain tumor types was described by McIntosh et al (1996).

1.2.3.2. Spatial configuration of β-glucan

The molecular structure of the OBG and the BBG polymeric chains has been described in detail and determined to be 90% composed of cellotriosyl and cellotetraosyl regions connected with (1→3)-β bonds (Wood 1984, MacGregor and Fincher 1993) with the rest being longer cellulosic regions. The configuration of the molecule in 3-

dimensional space is not yet known with certainty. Some intrinsic viscosity measurements suggest that it is probably a partially stiffened worm-like cylinder (Gomez et al 1997a).

Straight (1→4) cellulosic regions are extremely rigid sequences resistant to mechanical and chemical action (Sporns 2001). In addition, they can align and form microcrystalline cellulosic regions with strong hydrogen bonds, which makes them even more resistant to mechanical and chemical degradation. This alignment may be responsible for the gelation of β-glucan (Burkus and Temelli 1999). The (1→3)-β bond is responsible for a kink in the β-glucan structure (Woodward et al 1983). When present in a consecutive sequence, (1→3)-β-bond creates a regularly shaped helical structure which can form aggregates and gel (Zhang et al 1997)). The existence of consequtive (1→3)-β regions in BBG and OBG has not yet been proven with certainty, but a structural regularity of β-glucan caused by cellulosic regions (with i.e. 3 glucose units) regularly interrupted by a (1→3)-β bond may be the additional cause of β-glucan aggregation (Zhang et al 1997).

Due to the length of their cellulosic regions, barley β-glucan molecules are able to associate with each other and create micelles (Grim et al 1995) or gel (Burkus and Temelli 1999, Morgan and Ofman 1998). Varum et al (1992), using light scattering, confirmed that oat β-glucan also creates micelles. β-Glucan from *Poria cocos* (a kind of mushroom used in traditional Chinese herbal medicine), which is entirely composed of (1→3)-β-D-glucan, was also able to create molecular aggregates that dissolved completely in cadoxen (saturated CdO solution in 29% ethylenediamine) (Zhang et al 1997). The mechanism of aggregation was probably the formation of hydrogen bonds between regularly shaped random coil regions of β-glucan chains. Therefore, some aggregation between regularly shaped regions of BBG, if such regions exist, should not be precluded as a possible mechanism that enhances formation of micelles and gels (Fig. 1.1).

When heated above 60°C, micelles dissociate and individual molecules need more water for solvation. That could be the reason for the increased viscosity of BBG at low shear rates and at 70°C vs. 25°C reported by Gomez et al (1997b). At higher shear rates, individual molecules could align and viscosity at 70°C would drop below that at 25°C, which is expected. However, it is not clear if the rheometer used by Gomez et al (1997b) had Automatic Gap Control (adjustment of space between plates with the change in temperature) and if any precautions were taken to prevent evaporation of samples during heating to 70°C. The speculation by Gomez et al (1997b) that BBG aggregates at high temperatures is not likely. This argument was not supported by other scientists (Temelli 1994, Dawkins and Nnanna 1995) and needs further experimentation for confirmation.

1.2.3.3. Thermodynamic incompatibility of β-glucan

Incompatibility between BBG and milk proteins or starch was first described by Burkus (1996). Further experiments by Bansema (2000) attempted to establish the concentration-stability relationship between BBG and whey protein isolate (WPI). He found that a BBG-WPI mix was stable after two days of holding at refrigeration temperature when the β-glucan concentration was 0.25% and that of WPI was ≤5%. A higher concentration of either ingredient resulted in phase separation. However, a thermal treatment may cause instability even at 0.25% β-glucan concentration. More research is necessary to investigate β-glucan-protein compatibility under different pH and temperature conditions, at increased storage temperatures and with varying viscosities of

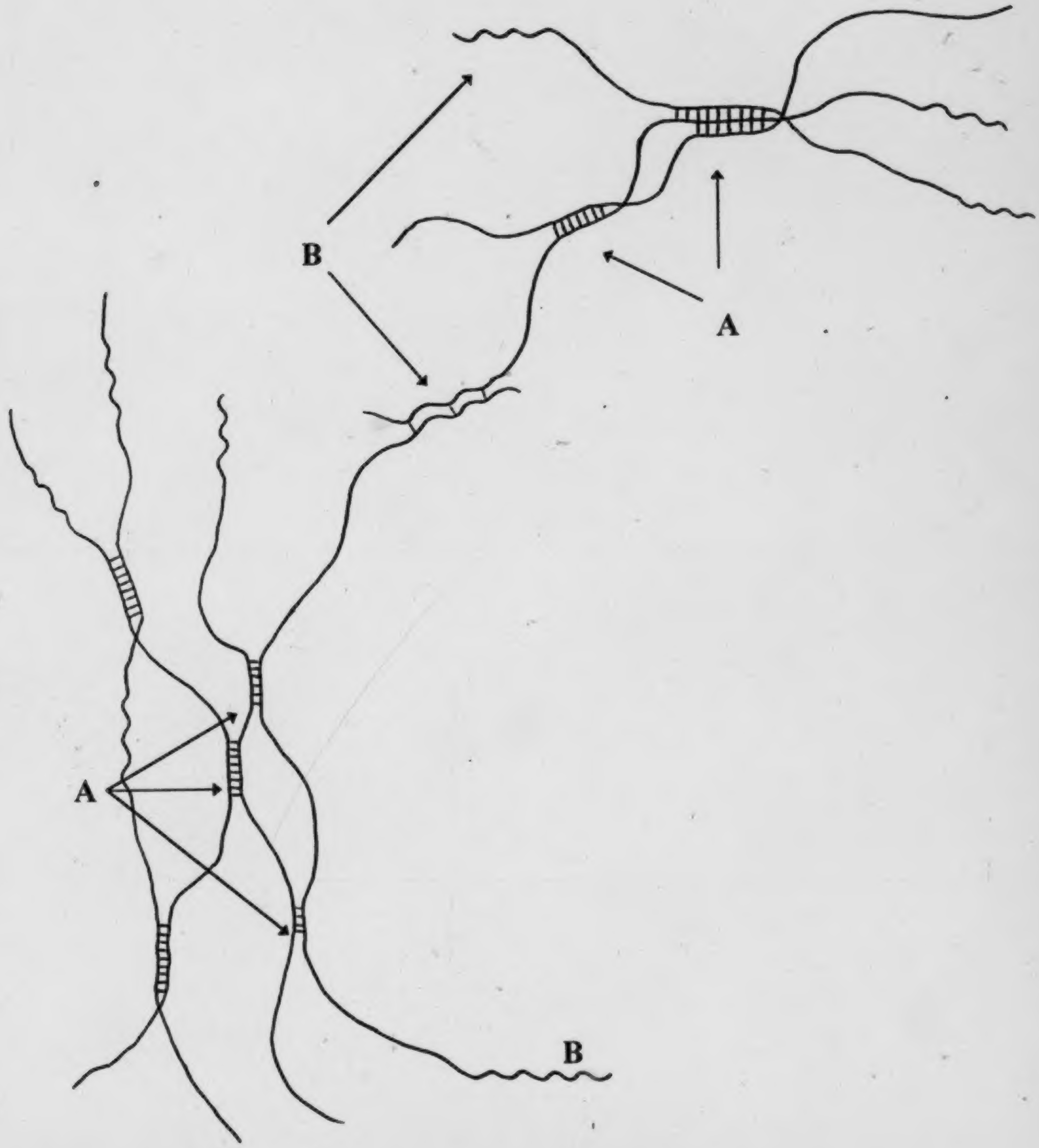

Figure 1.1. Possible structure of β-glucan micelles. β-Glucan chains could be aggregated into micelles through microcrystalline cellulosic regions (A) and micelles might be interconnected into flocs by regularly shaped regions (B).

BBG gums (low to high viscosity) and protein sources.

It seems that the components known to be precipitated by β-glucan have a globular structure. An important parameter in the evaluation of incompatibility is the time factor, but it has not been studied. An increase in viscosity delays sedimentation of a precipitated compound. Samples stored at refrigerator temperature have increased viscosity and, consequently, suspension of the incompatible compound is enhanced. The decrease in protein solubility and solvation does not have to be complete. Observation of partial insolubility of protein may require times longer than those employed in the quick laboratory experiments. The longer time frame associated with shelf life studies would provide sufficient time for proper incompatibility studies. However, care must be taken to exclude potential effects of all other factors, such as pH, temperature and bacterial contamination of samples. Incompatibility with other food components may be a major obstacle for food and beverage applications of β-glucan, but this problem can be offset by the suspending ability of β-glucan, adjusting component concentrations and proper choice of ingredients.

1.2.4. EXTRACTION OF β-GLUCAN

Successful large scale extraction of β-glucan has but a few major considerations - to obtain a high yield and high purity of stable HV β-glucan gum. At the same time, the process should be simple enough to ensure reproducibility. Cost effectiveness of the product, so that it can compete with other food hydrocolloids, is obviously a major concern.

A simplified β-glucan aqueous alkali extraction consists of the following steps:

- Barley milling to pass through a ≤500 μm screen.

- Flour mixed with water in the ratio ≥10:1.

- Alkali extraction with Na_2CO_3 at pH≈9 for ≥1 h.

- Separation of starchy insolubles using centrifugation.

- Proteins precipitated at pH≤4.5 and separated using centrifugation.

- Alcoholic precipitation of β-glucan from solution in ratio 1:1 (v/v).

- Drying of β-glucan gum.

- β-Glucan gum obtained containing ≥50% β-glucan.

Details of the extraction procedure, as it was carried out in this thesis, are described in Chapter 2.

1.2.4.1. Yield of BBG gum

A high yield of β-glucan can be achieved with one step aqueous alkali extraction (Wood et al 1977). Wood et al (1978) stated that a water:flour ratios in the range of 10:1 to 20:1 did not influence β-glucan yield after 30 min of extraction at 45°C. No difference in yield seems to be impossible because of mass distribution of soluble β-glucan. If one assumes that barley flour swells in water and keeps at least one part of water bound after extraction of β-glucan, then starting with a 10:1 vs. a 20:1 water:flour ratio results in 10% and 5%, respectively, of soluble β-glucan being retained with the starchy precipitate.

The yield and hydrolytic stability of β-glucan gum may be also influenced by a pretreatment of barley grains or flour, which is usually applied to stabilize the β-glucan extract by inactivating β-glucanase enzymes. Beer et al (1996) and Burkus and Temelli (1998) found that ethanol refluxing of flour lowered the yield of OBG and BBG gum, respectively, and did not deactivate β-glucanase. As well, Knuckles and Chiu (1999) tested different treatments as potential stabilizing methods (high pH, autoclaving, ethanol reflux, oven heat, 0.05 M $CaCl_2$, 0.1 N HCl and 5% trichloroacetic acid) and reported that none of these treatments completely inactivated β-glucanase. Extraction at pH 9 and 100°C resulted in the highest extraction yield and average MW of BBG (Knuckles and Chiu 1999). Zhang et al (1998) confirmed that increased extraction temperatures resulted in increased MW of OBG gum. Steaming of oat groats versus using raw and roasted groats for β-glucan extraction resulted in OBG with higher viscosity and intrinsic viscosity but with lower yield (Zhang et al 1998). Reduced yield could in part be caused by partial leaching of β-glucan during steam treatment of groats, which would form a low solubility film on the surface of groats upon drying or the initial leaching could have made ground particles more compact and harder to extract.

1.2.4.2. Purity of BBG gum

High purity product is always a goal of every extraction process. Further purification following extraction is always costly. The amount of impurities is primarily influenced by the extraction process parameters (Burkus, 1996).

Protein is considered to be the main impurity imparting a beige to brown color to BBG gum. Protein easily becomes incompatible with a hydrocolloid in the same solution and undergoes phase separation (Tolstoguzov 1991). When the gum is dissolved in water, proteins cause opalescence, which limits gum applications in certain products such as clear drinks. However, the lack of solution transparency may not be a problem in cloudy fruit beverages. Because of the low amount of protein present in BBG gum (usually <3%), the nutritional impact of protein is negligible.

Starch solubility is usually limited at extraction temperatures <55°C, but higher extraction pH increases its solubility through the lowering of the gelatinization temperature. Starch, as an impurity, is easily hydrolyzed with the addition of amylase-type enzymes. Thermostable α-amylase is the most often used enzyme (Bhatty 1993, 1995, Burkus and Temelli 1998). Once in the form of dextrins, hydrolyzed starch is more soluble and does not substantially influence the properties of BBG gum since it is present in a low amount. Non-hydrolyzed starch, even after cooking, partially keeps its globular form and may precipitate out of a BBG solution (Burkus 1996). The reason for precipitation may be gravitational separation enhanced by incompatibility similar to that between β-glucan and protein.

In general, pentosans make up the largest portion of impurities in BBG gum. The amount depends on extraction pH (Burkus 1996), but they should not be considered undesirable for a few reasons. They contribute to the viscosity of the BBG solution, which was clearly demonstrated by Bhatty et al (1991). They also seem to enhance the gelation of BBG gum as shown by Burkus and Temelli (1999), probably through making additional bridges between (1→4) regions. Cui et al (1999) extracted a mix of pentosans and β-glucan from a pearling-derived wheat fraction enriched in fiber. The gum gelled upon cooling at a 2% concentration. Gelation may not always be a desirable property, but the extraction process may be designed to extract the maximal amount of pentosans and thus increase the yield of total soluble fiber.

Ash is usually present in an amount <2%. Besides being extracted from the raw material, ash is normally produced during pH adjustment i.e. to pH 9 with Na_2CO_3 for the extraction of β-glucan and subsequent neutralization with HCl. If proteins are precipitated at pH 4.5 and the solution is again neutralized with NaOH or Na_2CO_3, additional NaCl is produced. However, NaCl is highly soluble, even in ethanol-water mixtures and the majority is separated by centrifugation with the ethanolic supernatant after the customary precipitation of β-glucan with ethanol. If drying is applied, all salt stays with the β-glucan gum. In small amounts, salt does not affect the solubility of BBG. In fact, salt may enhance the solubility of some proteins in food systems (i.e. globulins).

Estimation of gum purity depends on the quality of the β-glucan assay. Analysis according to McCleary and Glenny-Holmes (1985) using the Megazyme (Bray, Ireland) assay kits is routinely done in our laboratory. The following precautions are highlighted to minimize variability and possible sources of error.

McCleary and Glenny-Holmes (1985) recommend sample milling through a 500 μm screen. β-Glucan is present at higher concentration in coarse particles (Knuckles et al 1992) and extraction from the center of the coarser particles may be incomplete. Finer grinding is advisable. Extraction of β-glucan from the center of a large particle during 1.5 min of cooking time in glass tubes may be incomplete and lead to underestimation of the β-glucan content, especially if harder cultivars of oats and barley are being analyzed. It is better, for higher accuracy, to overcook the samples rather than to comply strictly with the recommended time. Special attention should be paid to the proper pipetting of the sample and the glucose standard since that may be the biggest source of error in β-glucan analysis. The sample should be pushed out of the tip slowly. Immersion depth for a 100 μL tip should not be >3 mm and eventual surplus fluid on the tip must be thoroughly cleansed . Finally, glucose standard readings may get lower with time. If the reason is deterioration of the glucose oxidase-peroxidase (GOPOD) reagent, then the influence on the glucose blank and samples is proportional. The other reason is microbial deterioration of the glucose standard giving lower glucose blank readings and overestimation of the β-glucan content. In our lab, absorbance readings for glucose blank are usually around 1.05, and any reading below 1.0 should be treated with suspicion. Buffer strength in GOPOD or sample can also affect results. For example, the stronger buffer utilized in starch analysis using the same Megazyme enzymes and standards regularly gives higher glucose blank absorbance.

1.2.4.3. Viscosity of BBG gum

If β-glucan is to be competitive in the hydrocolloid market, it should be of the HV type. At least, that is the current thinking based on demonstrated health benefits, which are mostly linked to viscosity. Dawkins and Nnanna (1995) found that from the standpoint of viscosity, oat β-glucan gum is comparable to locust bean gum and better than guar gum, while xanthan had much higher viscosity. Highly viscous β-glucan can be obtained from both oats and barley, although oats routinely outperforms barley (Wood et al 1991, Beer et al 1997b).

The FDA approved health claim for oat-rich products explicitly specifies ≥0.75 g of β-glucan per serving. HV β-glucan gum would be rarely used in concentrations >0.5% and for potential applications where it can be used as a thickener-stabilizer (salad dressing, mayonnaise, spreads, etc.) the typical serving size is quite small. The serving size is larger for beverages, usually ≥200 mL. Although neither β-glucan gum nor barley as a source of fiber has been approved for a health claim, about 0.5% gum with a purity of about 75% is necessary to satisfy the amount specified in the FDA claim. With HV gum, viscosity of such a beverage would be >50 mPa s even without other ingredients e. g. sugar. It could be too viscous for a beverage. In addition, viscosity in excess of about 10 mPa s may mask the flavor (Morris, 1989).

Some developmental work on a β-glucan rich beverage was performed by Bansema and Temelli (Bansema 2000) using a low viscosity (LV) BBG gum. A large enough quantity of a food-grade LV BBG gum were obtained at a pilot plant scale. It was easy to achieve the targeted 0.75 g per serving without any negative effect on flavor. The application of the same gum in low-fat sausages is also investigated (Morin 2001). LV gum can be prepared at approximately 3% concentration, which is necessary to adjust moisture content and achieve fat replacing properties, while handling of HV gum at concentrations >1% is almost impossible.

To elaborate further, the application of β-glucan in a product with a serving size of approximately 100 g, such as yogurt or pudding, where creaminess is an important quality attribute, may require the use of medium viscosity (MV) gum with viscosity in the range of 40-100 mPa s (1% w/w). Such gum can be obtained by adjusting extraction conditions to achieve controlled hydrolysis similar to Morgan and Ofman (1998), or by shearing β-glucan and lowering its MW.

In conclusion, β-glucan gums of different viscosities can be obtained from the same raw material with different extraction treatments (Burkus 1996), as desired for the final application. Additional considerations for the scale up of the extraction process are simplicity, cost and enough research to support potential applications of the β-glucan product.

1.2.4.4. Stability of BBG gum

Stability of β-glucan, in this case, is defined as constant viscosity over time, which is a desirable trait for a typical hydrocolloid. The main reason for the loss of viscosity is enzyme activity on the β-glucan chain, although viscosity loss due to agglomeration and precipitation was observed in our lab and also described in the Megazyme (Bray, Ireland) brochure for MW standards. The latter type of instability may be dealt with by reheating the β-glucan solution. Even though such instability may limit applications of BBG, it does not result in the physical loss of β-glucan. HV β-glucan is less prone to precipitate out of a solution, probably because of a larger hydration layer around the molecules or micelles, which keeps them dispersed. High viscosity itself also slows down such physical changes in a solution.

Viscosity instability due to enzyme activity is a much harder problem for several reasons. Historically, barley has been selected for high enzymatic activity and, as such, it has many β-glucanase enzymes to germinate the kernel quickly and transform it into malt or, actually, a new plant. These native enzymes work in the pH range 5-7 with optimal temperatures <40°C. Native β-glucanases are thermolabile and a thermal process above 60°C degrades them relatively easily (Ballance and Meredith 1976). The residual action of β-glucan solubilase at 60-70°C creates an additional problem in brewing. At such high temperatures, additional β-glucan is released from the cell wall matrix, but β-glucan hydrolases are already inactivated to a great extent. This "surviving" β-glucan passes through the beer brewing process with very little change. It is prone to gel and may clog filters or create haze in beer (Speers 1999).

On the other hand, when BBG gum is dissolved in boiling water, any native barley β-glucanase with residual activity after gum production is denatured. Native enzymes represent a much bigger problem during the extraction under mild temperature-pH conditions. Usually, barley flour is stirred into water. If extraction is carried out in the pH range 5-7 and temperature ≤50°C, these conditions are ideal for native enzymes. In general, a slurry of barley flour in distilled water has a pH of 6.2-6.5. Even if the extraction is carried out at ≥55°C, just mixing of flour with water at room temperature with subsequent heating to 55°C would allow sufficient time for hydrolases to cleave β-glucan into LV type. Therefore, preheating the water to the desired temperature prior to mixing of flour will minimize such changes and give a consistent product.

Similarly, preadjustment of pH is a must. Extraction at pH 7 was reproducible only if water was premixed with sufficient Na_2CO_3 to keep the pH at 7 after mixing of flour with water (Burkus 1996). The amount of Na_2CO_3 was determined experimentally and overshooting in the mildly basic pH region (pH 7-9) was less harmful than undershooting. Following these steps (mixing flour into preheated water at desired pH), it was possible to obtain a reproducible gum viscosity of approximately 40 mPa s for 1% (w/w) concentration (Burkus 1996). Conversely, mixing the flour with water, adjusting the pH to 7 and subsequently heating may look logical to an inexperienced researcher, but resulted in BBG gum viscosity of 10-15 mPa s regardless of flour handling speed.

Higher extraction pH may suffice to diminish enzyme activity, but then the extraction time is extended for the time necessary to heat the sample from room temperature to the desired point and this time may vary and affect reproducibility. Again, mixing flour into preheated water at the desired pH would minimize variability. So, if extraction parameters are strictly controlled, β-glucanase activity native to barley can be controlled or eliminated.

Microbial enzymes, inherited from environmental pollution, are normally present on the barley kernels. Some of them are β-glucanases and some of these β-glucanases are glycoenzymes, which are often heat stable (Wood et al 1989). These enzymes can survive extraction conditions and thus can be present in the dried BBG gum. When the gum is later hydrated at a favorable pH, the enzymes start to cleave β-glucan. The resulting viscosity drop may be small, but measurable over time if BBG gum is used in products that have a shelf life of a few months. Wood et al (1989) suspected residual microbial glucanase activity to be a factor in the degradation of their pilot plant gum. Thus, β-glucan extraction should be designed in a way that greatly reduces, if not completely inactivates this group of enzymes.

Complete enzyme inactivation seems difficult to achieve. Ethanol reflux of barley flour was not sufficient (Burkus and Temelli 1998), while dry heating of kernels was even less effective resulting in lower yields (Zhang et al 1998). Wet heat combined with high temperature - high pH extraction resulted in the highest level of β-glucanase inactivation (Zhang et al 1998). The use of chemicals for enzyme inactivation is problematic because they always remain in the final BBG gum product and effective ones are not desired in food applications of the gum. While $CaCl_2$ was partially effective in inactivating β-glucanase (Knuckles and Chiu 1999), recent application of some food grade chemicals for β-glucan extraction looks promising (Vasanthan 2001).

1.2.4.5. Rheological properties of β-glucan

Being a hydrocolloid, β-glucan may form highly viscous pseudoplastic solutions with a flow behavior index $n<<1$ if it is of the high viscosity type (Autio et al 1987, Bhatty 1995, Burkus and Temelli 1998), but even HV β-glucan at concentrations $\leq 0.25\%$

behaves as a Newtonian fluid (Autio et al 1987, Burkus and Temelli 1998). Low viscosity gums exhibit basically Newtonian flow behavior at concentrations ≤1% (Burkus and Temelli 1998). In the presence of other solutes, such as salt and sugar, β-glucan solutions may have increased viscosity (Autio et al 1987, Dawkins and Nnanna 1995, Bansema 2000), but the increase is concentration dependent and the order of hydration may affect the final solution viscosity (Burkus 1996).

Gelation of LV BBG was described by Burkus and Temelli (1999) and Morgan and Ofman (1998). The firmness of the network and the speed of network formation were concentration and viscosity dependent. HV BBG could not set into a gel even after extended periods of time despite increasing the BBG concentration to 3.8% (Burkus and Temelli 1999). Higher gum concentrations were impossible to work with in the case of HV BBG gum. Gels formed by BBG were reversible and melted at 58-60°C. Gel-like behavior of hydrolyzed OBG was observed by Doublier and Wood (1995).

Thixotropy is a decrease in viscosity under constant shear rate over a period of time (Muller 1973). It is the consequence of three-dimensional network destruction under the influence of shear over an extended period of time (Schramm 1994). A thixotropic liquid is defined by its potential to have its gel structure reformed, whenever the substance is allowed to rest for an extended period of time and the gel to sol and sol to gel transition is reproducible any number of times (Schramm 1994).

Autio et al (1987, 1992), Wood et al (1989) and Wikstrom et al (1994) did not observe thixotropy in the behavior of OBG gum. However, Linemann and Kruger (1997) reported thixotropic behavior of β-glucan isolated from beer. Thixotropy of that β-glucan was temperature dependent.

Yield point is exhibited by samples with inner structure, which at small shear stress undergo an elastic change. Such a sample resembles a solid material for any value of shear stress that does not surpass the critical level named “yield value” or “yield point” (Schramm 1994). Beyond that level of shear stress, the sample starts to flow. The proper measurement of yield point is much more accurate using a Controlled Stress rheometer than is possible with a Controlled Rate rheometer (Schramm 1994).

Viscoelastic properties of polymer solutions are important because besides viscous flow, they also exhibit elements of solid-like behavior. These two ways of response to stress, solid-like and liquid-like, are usually characterized by **G’** and **G’’** moduli. During oscillatory deformation, the ratio of in-phase stress to the amount of shear is the storage (elastic) modulus **G’**, while the ratio of out-of-phase stress to shear is the loss (viscous) modulus **G’’** (Robinson et al 1982). These two moduli are related to complex viscosity η^* according to Eq. 1.1 (Autio 1988)

$$\eta^* = \frac{\sqrt{G'^2 + G''^2}}{\omega} \qquad (1.1)$$

where ω is the frequency of oscillation and η^* is also referred to as ‘dynamic viscosity’ (Morris 1989). For entangled polysaccharide solutions (and non-entangled dilute solutions), the plot of η^* vs. ω is closely superimposable on the plot of viscosity η vs. shear rate when measured at equivalent numerical values of ω (rad/s) and shear rate (s^{-1}) (Morris 1989). This rule is not applicable to xanthan solutions, which behave as a weak gel when G’ exceeds G’’ at all frequencies (Morris 1989). Typical rheological responses of a diluted and concentrated polysaccharide solution and a strong gel are shown in Figure 1.2 (Morris 1989).

Page 24 has been removed due to copyright restrictions. The information removed was Figure 1.2. titled "**Figure 1.2.** Frequency dependence (ω) of storage modulus G' (– – –), loss modulus G'' (- - -) and dynamic viscosity η* (——) for polysaccharide systems (Morris 1989); and presented as Fig. 4. in Morris, E.R. 1989. Polysaccharide solution properties: origin, rheological characterization and implications for food systems. Pages 132-163 in: Frontiers in Carbohydrate Research-1: Food Applications. R.P. Millane, J.N. BeMiller, and R. Chandasekaran, eds, Elsevier Applied Science. New York, N.Y.

Autio (1988) observed that 0.35-0.66% OBG solutions behaved as concentrated solutions in Figure 1.2. The addition of sugar resulted in overlap of G' and G'' at a lower frequency. The addition of salt into a 0.345% (w/w) OBG solution caused a decrease in G' at frequencies <2 Hz (Wikstrom et al 1994). Concentration dependence of the frequency at which G' and G" overlapped was shown by Robinson et al (1982) for guar gum. Doublier and Wood (1995) reported gel-like behavior of partially hydrolyzed oat gum when G' was higher than G" at frequencies below 3 rad/s. Cui et al (1999) observed a thermally reversible gel network development in a 2% solution of wheat non-starch polysaccharides upon cooling through monitoring G' and G'' over time.

Current understanding of the network formation and gelation behavior of BBG gum is quite limited. Better characterization of the above rheological properties and how they are affected by various processing parameters is essential for optimal process design as well as successful product applications of BBG.

1.2.4.6. Applications of β-glucan

Although extracted on a commercial scale, neither barley nor oat β-glucan gum is used as an ingredient in food products, mainly due to the high price of high purity gums (Wood and Beer 1998). Oatrim®, an extract of hydrolyzed oat flour containing 1-10% β-glucan, is used as a fat replacer in some types of low fat milk products (Pszczola 1996). Oatrim is used in several of ConAgra's Healthy Choice products-hot dogs, bologna, cheese, and 96 percent fat-free ground beef. Smaller companies are using the fat-replacer in baked products such as muffins and cookies and in chocolate candy (Grisamore 2000).

There are plans by Quaker Oats and Novartis Consumer Health to make fortified products enriched with oat β-glucan (Swientek 2000). It is also possible to find some types of β-glucan concentrate offered over the Internet as dietary supplements (Wheeler 2001). At one time, it was possible to obtain approximately 15% and >65% BBG powder from Koster Keunen Inc. (Watertown, CT).

With advances in technology, there is no doubt that β-glucan production will be simplified, yields increased and byproducts better utilized, leading to successful and cost effective barley fractionation. There are already major advances being made in that direction (Vasanthan 2001).

1.4. REFERENCES

Autio, K. 1988. Rheological properties of solutions of oat β-glucans. Pages 483-488 in: Gums and Stabilizers for the Food Industry 4. G. O. Phillips, P. A. Williams and D. J. Wedlock, eds, IRL: Washington, D C.

Autio, K., Myllymaki, O., and Malkki, Y., 1987. Flow properties of solutions of oat β-glucans. J. Food Sci. 52:1364-1366.

Autio, K., Malkki, Y., and Virtanen, T. 1992. Effects of processing on the microstructure of oat (*Avena sativa*) bran concentrate and the physicochemical properties of isolated β-glucans. Food Structure 11:47-54.

Ballance, G. M., and Meredith, W. O. S. 1976. Purification and partial characterization of an endo-β-1,3-glucanase from green malt. J. Inst. Brew. 82:64-67.

Bansema, C. 2000. Development of a Barley β-Glucan Beverage With and Without Whey Protein Isolate. M. Sc. Thesis. University of Alberta, Edmonton, AB.

Beer, M. U., Arrigoni, E., and Amado, R. 1996. Extraction of oat gum from oat bran: effects of process on yield, molecular weight distribution, viscosity and (1→3)(1→4)-β-D-glucan content of the gum. Cereal Chem. 73:58-62.

Beer, M. U., Wood, P.J., Weisz, J. and Fillion, N. 1997a. Effect of cooking and storage on the amount and molecular weight of (1→3)(1→4)-β-D-glucan extracted from oat products by in vitro digestion system. Cereal Chem. 74:705-709.

Beer, M.U., Wood, P.J., and Weisz, J. 1997b. Molecular weight distribution and (1→3) (1→4)-β-D-glucan content of consecutive extracts of various oat and barley cultivars. Cereal Chem. 74:476-480.

Bhatty, R. S. 1993. Extraction and enrichment of (1→3)(1→4)-β-D-glucan from barley and oat brans. Cereal Chem. 70:73-77.

Bhatty, R. S. 1995. Laboratory and pilot plant extraction and purification of β-glucans from hull-less barley and oat bran. J. Cereal Sci. 22:163-170.

Bhatty, R. S. 1999. The potential of hull-less barley. Cereal Chem.76:589-599.

Bhatty, R. S. 2000. An overview of the 8th international barley genetics symposium. Barley Country 9(4):14-15.

Bhatty, R. S., and Rossnagel, B. G. 1997. Zero amylose lines of hull-less barley. Cereal Chem. 74:190-191.

Bhatty, R. S., MacGregor, A. W., and Rossnagel, B. G. 1991. Total and acid soluble β-glucan content of hulless barley and its relationship to acid extract viscosity. Cereal Chem. 68:221-227.

Burkus, Z. 1996. Barley β-glucan: Extraction, Functional Properties and Interaction with Food Components. M. Sc. Thesis. University of Alberta, Edmonton, AB.

Burkus, Z., and Temelli, F. 1998. Effect of extraction conditions on yield, composition and viscosity stability of barley β-glucan gum. Cereal Chem. 75:805-809.

Burkus, Z., and Temelli, F. 1999. Gelation of barley β-glucan concentrate. J. Food Sci. 64:198-201.

Causey, J. L., McKeehen, J. D., Slavin, J. L., and Fulcher, R. G. 1998. Differential modulation of human macrophages by cereal cell wall β-glucans and phenolic acids. Paper # 26. Presented at the AACC Annual Meeting, Minneapolis, MN. Sept. 13-17.

Collins, B. 1998. Phytochemicals from oats…Where are we now and where are we going? Cereal Science Symposium - Updates and Inovations II. The Oat Story… Strategies for Successful, Inovative Functional Food Developments. Alberta Section of CIFST, Edmonton, AB, April 27.

Cui, W., Wood, P. J., Weisz, J., and Beer, M. U. 1999. Nonstarch polysaccharides from preprocessed wheat bran: Carbohydrate and novel rheological properties. Cereal Chem. 76:129-133.

Dawkins, N. L. and Nnanna, I. A. 1995. Studies on oat gum [(1→3, 1→4)-β-D-glucan]: composition, molecular weight estimation and rheological properties. Food Hydrocol. 9:1-7.

Doublier, J. L. and Wood, P. J. 1995. Rheological properties of aqueous solutions of (1→3) (1→4)-β-D-glucan from oats (*Avena sativa* L.). Cereal Chem. 72:335-340.

Estrada, A., Yun, C-H., Van Kessel, A., Li, B., Hauta, S., and Laarveld, B. 1997. Immunomodulatory activities of oat β-glucan in vitro and in vivo. Microbiol. Immunol. 41:991-998.

FAO. 2000. FAO Statistical Databases at http://apps.fao.org Agriculture: Agricultural Production-Crops Primary.

FDA. 1997. Food Labeling; Health Claims; Oats and Coronary Heart Disease; Final Rule Federal Register Doc. 97-1598, filed 1-22-97.

Grimm, A., Kruger, E., and Burchard W. 1995. Solution properties of β-D-(1,3)(1,4)-glucan isolated from beer. Carbohydr. Polym. 27:205-214.

Gomez, C., Navarro, A., Manzanares, P., Horta, A., and Carbonell, J.V. 1997a. Physical and structural properties of barley (1→3),(1→4)-β-D-glucan II. Viscosity, chain stiffness and macromolecular dimensions. Carbohydr. Polym. 32:17-22.

Gomez, C., Navarro, A., Garnier, C., Horta, A., and Carbonell, J. V. 1997b. Physical and structural properties of barley (1→3),(1→4)-β-D-glucan III. Formation of aggregates analyzed through its viscoelastic and flow behavior. Carbohydr. Polym. 34:141-148.

Gosain, K. 1996. Long Term Effects of Barley Bread Products on Metabolic Control of Non Insulin Dependent Diabetes Mellitus. M. Sc. Thesis, University of Alberta, Edmonton, AB.

Grisamore, S. 2000. In: Two Thumbs up for Oatrim. www.food.us.rhodia.com/brochures/oatrim/page2.asp

Knuckles, B. E., Chiu, M. M., and Betschart, A. A. 1992. β-Glucan enriched fractions from laboratory scale dry-milling and sieving of barley and oats. Cereal Chem. 69:198-202.

Knuckles, B.E., and Chiu, M-C.M. 1999. β-Glucanase activity and molecular weight of β-glucans in barley after various treatments. Cereal Chem. 76:92-95.

Linemann, A., and Kruger, E. 1997. Relationships between structure and characteristics of β-glucan in beer production. Part 1: Properties in dissolving β-glucan out of beer. Brauwelt International 1997(IV):344-351.

MacGregor, A. W., and Fincher, G. B. 1993. Carbohydrates of the barley grain. Pages 73-130 in: Barley: Chemistry and Technology. A. W. MacGregor and R. S. Bhatty, eds, Am. Assoc. Cereal Chem. Inc.: St. Paul, MN.

McCleary, B. V. and Glennie-Holmes, M. 1985. Enzymic quantification of (1→3)(1→4)-β-D-glucan in barley and malt. J. Inst. Brew. 91:285-295.

McIntosh, G. H., Le-Leu, R. K., Royle, P. J., and Young, P. G. 1996. A comparative study of the influence of differing barley brans on DMH-induced tumors in male Sprague-Dawley rats. J. Gastroenter. Hepatol. 11:113-119. Reported in: Bhatty, R. S. 1999. The potential of hull-less barley. Cereal Chem. 76:589-599.

Morgan, K. R., and Ofman, D. J. 1998. Glucagel, a gelling β-glucan from barley. Cereal Chem. 75:879-881.

Morin, L. 2001. Development of a Reduced-Fat Breakfast Sausage Utilizing Barley β-Glucan as a Fat Replacer. M. Sc. Thesis. University of Alberta, Edmonton, AB.

Morris, E.R. 1989. Polysaccharide solution properties: origin, rheological characterization and implications for food systems. Pages 132-163 in: Frontiers in Carbohydrate Research-1: Food Applications. R.P. Millane, J.N. BeMiller, and R. Chandasekaran, eds, Elsevier Applied Science. New York, N.Y.

Muller, H. G. 1973. An Introduction to Food Rheology. William Heinemann Ltd., London, UK.

Newman, R. K. and Newman, C. W, 1991. Barley as a food grain. Cereal Foods World 36:800-805.

Pszczola, D. E. 1996. Oatrim finds application in fat-free, cholesterol-free milk. Food Technol. 50:80-81.

Robinson, G., Ross-Murphy, S. B., and Morris, E. R. 1982. Viscosity-molecular weight relationships, intrinsic chain flexibility, and dynamic solution properties of guar galactomannan. Carbohydr. Res. 107:17-32.

Schramm, G. 1994. A Practical Approach to Rheology and Rheometry. Gebrueder HAAKE GmbH: Karlsruhe, Germany.

Speers, A. 1999. Personal communication. Department of Food Science and Technology Daltech, Dalhousie University, Halifax, NS.

Sporns, P. 2001. Personal communication. University of Alberta, Edmonton, AB.

Statistics Canada. 2000. Field crop reporting series. Vol. 79(8). Statistics Canada, Ottawa, ON.

Swientek, B. 2000. Raising Nutrition to New Heights. www.nutrasolutions.com/articles/current/junealtus.html

Temelli, F. 1994. Functional properties of β-glucan enriched barley fractions. Abstract # P325. Presented at the 37th Annual Conference of CIFST, Vancouver, BC. May 15-18.

Tolstoguzov, V. B. 1991. Functional properties of food proteins and role of protein-polysaccharide interaction. Food Hydrocol. 4:429-468.

Vasanthan, T. 2001. Personal communication. University of Alberta, Edmonton, AB.

Vasanthan, T. and Bhatty, R. S. 1995. Starch purification after pin milling and air classification of waxy, normal, and high-amylose barleys. Cereal Chem. 72:379-384.

Varum, K. M., Smidsrod, O., and Brant, D. A. 1992. Light scattering reveals micelle-like aggregation in the (1→3),(1→4)-β-D-glucan from oat aleurone. Food Hydrocol. 5:497-511.

Wheeler D. 2001. www.davidwheeler.com/index2.html

Wikstrom, K., Lindahl, L., Andersson, R., and Westerlund, E. 1994. Rheological studies of water-soluble (1→3), (1→4)- β-D-glucans from milling fractions of oats. J. Food Sci. 59:1077-1080.

Wood, P.J. 1984. Physicochemical properties and technological and nutritional significance of cereal β-glucans. Pages 35-78 in: Cereal Polysaccharides in Technology and Nutrition. V. F. Rasper, ed. Am. Assoc. Cereal Chem. Inc.: St. Paul, MN.

Wood, P. J., Paton, D., and Siddiqui, I. R. 1977. Determination of β-glucan in oats and barley. Cereal Chem. 54:524-533.

Wood, P. J., Siddiqui, I. R., and Paton, D. 1978. Extraction of high viscosity gums from oats. Cereal Chem. 55:1038-1049.

Wood, P.J., Weisz, J., Fedec, P., and Burrows, V. D. 1989. Large scale preparation and properties of oat fractions enriched in (1→3)(1→4)-β-D-glucan. Cereal. Chem. 66:97-103.

Wood, P. J., Weisz, J., and Mahn, W. 1991. Molecular characterization of cereal β-glucans II, size exclusion chromatography for comparison of molecular weight. Cereal Chem. 68:530-536.

Wood, P. J., and Beer, M. U. 1998 Functional oat products. Pages 1-37 in: Functional Foods - Biochemical and Processing Aspects. Mazza, G., ed. Technomic Publishing Company, Inc.: Lancaster, PA.

Woodward, J. R., Fincher, G. B. and Stone, B. A. 1983. Water soluble (1→3)(1→4)-β-D-glucans from barley (*Hordeum vulgare*) endosperm. II. Fine structure. Carbohydr. Polym. 3: 207-225.

Zhang, L., Ding, Q., Zhang, P., Zhu, R., and Zhou, Y. 1997. Molecular weight and aggregation behavior in solution of β-D-glucan from *Poria cocos* sclerotium. Carbohydr. Res. 303:193-197.

Zhang, D., Doehlert, D. C., and Moore W. R. 1998. Rheological properties of (1→3),(1→4)-β-D-glucans from raw, roasted and steamed oat groats. Cereal Chem. 75:433-438.

Zheng, G. H., Han, H. L., and Bhatty, R. S. 1998. Physicochemical properties of zero amylose hull-less barley starch. Cereal Chem. 75:520-524.

2. PILOT PLANT AND LABORATORY SCALE EXTRACTION OF BARLEY β-GLUCAN GUM[1]

2.1. INTRODUCTION

Extraction of stable and highly viscous barley β-glucan (BBG) gum was one of the main goals of Burkus (1996). It was reported that BBG extracts could be stabilized either by boiling at the extraction pH of 9 or 10, or by boiling with thermostable α-amylase following ethanol refluxing of flour. Refluxing alone was not sufficient to stabilize the BBG gum regardless of the extraction pH. Extraction at pH 10 was considered to be too high and detrimental for the viscosity of BBG gum, probably because of the influence of high pH on (1→3)-β bonds (McCleary and Codd 1991). As well, high pH resulted in an increase in the level of impurities in the gum product. Extraction of BBG from barley flour at boiling temperature was not chosen since preservation of native ungelatinized starch was another goal. Waxy barley starch may have potential use as a food ingredient (Bhatty and Rossnagel 1997, Zheng et al 1998) and thus have a significant impact on the cost-effectiveness of larger scale extractions. In addition, following purification with α-amylase, the discarded ethanol supernatant from β-glucan precipitation would contain significant amounts of maltose and dextrins, resulting in a very high biological oxygen demand in the case of scale up without a government permit for ethanol recovery.

Unfortunately, Burkus (1996) did not add any preservative to the extracts during viscosity stability testing, which limited conclusions about the effectiveness of applied stabilization methods over 24 h. The chosen water:flour ratio of 10:1 was somewhat low since high pH extracts were very thick and it was hard to separate impurities by centrifugation. Inefficiency of ethanol refluxing of flour, when applied as the only stabilization method, was later confirmed by Burkus and Temelli (1998).

---

[1] A version of this chapter is to be submitted to Cereal Chemistry for consideration for publication.

Wood et al (1989) extracted oat β-glucan at the pilot plant (PP) scale using pH 10 and 33-35°C for 30 min as extraction conditions. The raw material, oat bran, was refluxed with 75% ethanol for 4 h, which Wood et al (1989) considered to be sufficient to stabilize the extract. The use of untreated bran resulted in an extract, which lost 24% of its viscosity in 1 h. Viscosity loss of extract from refluxed bran during the pilot plant procedure was in part ascribed to phenolics extracted at pH 10. As well, Wood et al (1989) measured a significant drop in the viscosity of the PP extract after each centrifugation step; extract obtained at the laboratory (LAB) scale did not exhibit this loss in viscosity. This finding demonstrated the influence of shear on the molecular weight of β-glucan. Viscosity loss in extract from deactivated bran was in part attributed to heat resistant microbial enzymes. Although the 1 h test did not show any significant change in viscosity, a 24 h test, as performed by Burkus (1996), would provide a better indication of the decrease in extract viscosity.

Bhatty (1995) carried out pilot plant extraction of β-glucan from oat and barley using 0.25 *N* NaOH. The β-glucan gum products had lower viscosities than those obtained by Wood et al (1989). β-Glucan hydrolysis by extracted phenolics (Wood et al 1989) or very high pH (probably pH>11) could be the reason for the lower viscosity (Burkus and Temelli 1998, Bhatty 1999). Bhatty (1995) tested β-glucanase activity in bran using the Megazyme malt β-glucanase assay procedure. The negative result obtained was due to the low sensitivity of the method for non-malt material (McCleary 1995). In fact, only viscosity tests can reliably detect low enzymatic activity (McCleary 1995). Extractability of β-glucan was high, but recovery from barley extracts was <77%. Shorter chains of digested β-glucan would have increased solubility and would be carried out with the ethanolic supernatant stream from precipitation. Bhatty (1995) did not test the extracts for viscosity stability nor did he report any equipment shear effects on the β-glucan viscosity.

Although not at the pilot plant scale, Morgan and Ofman (1998) attempted a unique approach to obtain β-glucan from untreated barley flour. Aqueous extraction was carried out at 25-55°C without pH adjustment for 0.5 to 6 h; precipitation was performed by freezing at -10°C with subsequent thawing. Precautions were not undertaken to minimize β-glucan hydrolysis. Yields of β-glucan after 0.5 h extraction (<3%) were lower than yields obtained by Burkus (1996) (>4%) from barley with similar β-glucan content (7% vs. 6.8% β-glucan in Burkus 1996). Enzymatic activity differs with barley cultivar and the barley cultivar used by Morgan and Ofman (1998) may have had low enzyme activity. Freeze-thaw stability experiments conducted in our laboratory using 1% β-glucan solutions at approximately -15°C could not confirm the findings of Morgan and Ofman (1998), although it cannot be precluded that freezing at higher temperature of −10°C is incomplete and causes β-glucan to agglomerate and precipitate. However, they may not have disclosed all the experimental details due to a pending patent application. Their statement that "on cooling <55°C, solutions containing >0.5% Glucagel set to form a soft gel, regardless of molecular weight" (Morgan and Ofman 1998) is not supported by the experimental procedure. Based on Burkus and Temelli (1999), β-glucan solution at such a low concentration would not be expected to form a gel within a reasonable period of time.

A critical review of previous studies (Wood et al 1989, Bhatty 1995, Burkus 1996) leads to the following conclusions for future extraction work: high extraction pH at ≤55°C should be applied to extract high viscosity (HV) β-glucan and preserve starch as much as possible. Very high extraction pH effectively inactivates anything active, including enzymes, but pH≥10 seems to be too high for β-glucan extraction because of the probability that at elevated temperatures it may lead to β-glucan digestion either by cleavage of (1→3) bonds (McCleary and Codd 1991) or by the extraction of phenolics that may also cleave β-glucan (Wood et al 1989). Proteins are also less stable at such high pH levels and may lose some of their nutritive value due to amino acid degradation (Hurrel and Finot 1985). In addition, starch granules swell and become more soluble at high pH, which would decrease extract purity. Heating nearly to boiling should be applied to stabilize the β-glucan extract. It may be combined with high pH to ensure enzyme inactivation. Barley flour should be mixed into preheated aqueous alkali to instantly achieve high extraction pH and minimize β-glucanase activity. Viscosity stability tests should be continued for at least 7 days so that results would be easier to extrapolate to a longer shelf life period for potential food applications.

Design of a cost effective extraction of β-glucan requires data about the composition of all fractions obtained after β-glucan extraction. However, none of the previous studies reported the composition of starch- and protein-rich residues, nor long term viscosity stability of the β-glucan gum product. As well, for detailed studies of BBG gum behavior under different conditions and for the testing of its potential future food applications, a sufficient amount of food-grade BBG gum is necessary. Therefore, the objectives of this study were to obtain HV BBG gum at laboratory and pilot plant scale and to compare the yield, purity and viscosity stability of the obtained gums.

2.2. MATERIALS AND METHODS

2.2.1. Materials

Bly Blend waxy hulless barley (a mixture of two experimental cultivars, SB89528 and SB89497) with 7.16% β-glucan (as is) was provided by Dr. J. Helm, Alberta Agriculture, Food and Rural Development, Lacombe, AB. All materials used for PP extraction were food grade. Sodium carbonate was from BDH Inc. (Toronto, ON), HCl was purchased from Fisher Scientific Co. (Nepean, ON) and ethanol (99.9%) was from Commercial Alcohols Inc. (Brampton, ON). Termamyl 120 LN, a thermostable α-amylase (E.C. 3.2.1.1.) from *Bacillus licheniformis* was kindly provided by Novo Nordisk BioChem, Inc. (Toronto, ON).

2.2.2. Extraction of β-glucan

For LAB extraction, whole kernels were first crushed in a Magic Mill Model III Plus (Magic Mill, Salt Lake City, UT) and then finely ground to <500 μm using a Udy Cyclone Sample Mill (model 3010-30, Udy Corp., Fort Collins, CO). For PP extraction, barley was pin-milled in a Contraplex wide-chamber A250 mill (Alpine Corp., Augsburg, Germany) to pass through a 425 μm (40 mesh) screen. During pin-milling, the door side disk was revolving at 5600 rpm, whereas the housing side was rotating at 3300 rpm. The feed rate was 90 kg/hr.

The laboratory extraction procedure was based on Wood at al (1978) with major modifications as described in Burkus and Temelli (1998). The extraction protocol involved a combination of boiling and Termamyl treatments from Burkus (1996). Batch size was 200 g of barley flour. Figure 2.1 depicts the extraction protocol for both LAB and PP extraction.

Pilot Plant extraction of 200 kg barley flour was carried out at POS Pilot Plant Corp. (Saskatoon, SK). Original extraction plan was modified due to different capacities and separation ability of available equipment, as shown in Figure 2.1. Starch separation had to be done in two-steps, first using a Westfalia decanter EC3 (Centrico, Northvale, NJ) (500 kg/hr, back drive speed 20-23 rpm, back pressure 117-138 kPa) and then with a Westfalia desludger DC2 (400 kg/hr, back pressure 297-331 kPa). Protein was also separated on the Westfalia DC2 desludger (500 kg/hr, back pressure 241-276 kPa). Ethanol precipitation was done in an explosion proof area. After standing for 5 h with intermittent agitation, the slurry was first centrifuged using a Westfalia EC3 decanter (800 kg/hr, back drive speed 20-24 rpm, 110-138 kPa), then crude gum was mixed with 200 L of ethanol and centrifuged using a Bird decanter EC2 (300 kg/hr) (Bird Machine, S. Walpole, MA). Washing with 200 L of ethanol was repeated. Washed β-glucan gum was vacuum dried at 3.1 kPa and 60°C and milled to pass a 20 mesh screen.

Starch- and protein-rich residues from PP extraction were designated as follows: heavy fractions 1 and 2 (HF1 and HF2) for starch-rich phases, whereas HF3 was used for the protein-rich precipitate after pH adjustment to 4.5.

2.2.3. Chemical analyses

Samples were dried overnight at 80°C (McCleary and Glennie-Holmes 1985), dry weight recorded and samples were left 72 h on the bench at room temperature to

LABORATORY EXTRACTION

Barley flour, 200 g
⇩
Mix with 2400 mL water 55°C, 2.5 g of Na_2CO_3, adjust pH 9.4
⇩
Extract 1 h at 53-55°C with stirring
⇩
Centrifuge, 17,600xg, 25 min
⇩ ⇨ Starchy residue
Supernatant, heat to ~90°C, hold 1 h (enzyme inactivation)
⇩
Adjust pH to 6.5 with HCl, add 140 mg $CaCl_2$, add Termamyl (1 mL), incubate 1h at 90°C with stirring
⇩
Adjust pH to 4.5 (HCl)
⇩
Centrifuge, 17,600xg, 25 min
⇩ ⇨ Proteinaceous residue
Adjust pH to ~7 (HCl)
⇩
Precipitate with 1:1 vol. abs. EtOH, leave overnight
⇩
Centrifuge 3,300xg, 10 min
⇩
Gum pellet, resuspend in 150 mL abs. EtOH with Polytron at speed 1
⇩
Filter on Whatman No. 1 under vacuum, wash with 50 mL ethanol
⇩
Air dry, fumehood, then overnight at 80°C
⇩
Pulverize, mortar and pestle

PILOT PLANT EXTRACTION

Barley flour, 200 kg
⇩
Mix with 2400 L water 55°C, 2.5 kg of Na_2CO_3, adjust pH 9.4
⇩
Extract 1 h at 53-55°C with stirring
⇩
Centrifuge, Westfalia decanter
⇩ ⇨ Starchy residue (HF1)
Centrifuge, Westfalia desludger
⇩ ⇨ Starchy residue (HF2)
Supernatant, heat to ~90°C, hold 1 h (enzyme inactivation)
⇩
Adjust pH to 6.5 with HCl, add 140 g $CaCl_2$, add Termamyl (200 mL), incubate 1hr at 90°C with stirring
⇩
Adjust pH to 4.5 (HCl)
⇩
Centrifuge, Westfalia desludger
⇩ ⇨ Proteinaceous residue (HF3)
Adjust pH to ~7 (HCl)
⇩
Precipitate with 1:1 vol. abs. EtOH, leave 5 hr
⇩
Centrifuge, Westfalia decanter
⇩
Crude gum, resuspend with 200 L abs. EtOH, in-line mixer
⇩
Centrifuge, Bird decanter
⇩
Washed gum, repeat washing and centrifugation
⇩
Washed gum 2, vacuum tray dryer (3.1 kPa, 60°C)
⇩
Mill to pass 20 mesh screen

Figure 2.1. Flow chart of laboratory and pilot plant extraction of barley β-glucan from whole barley flour.

equilibrate. Then, the moist weight was recorded and the weight difference was calculated as the moisture content. All analyses were performed after moisture determination. β-Glucan content of all samples was determined according to McCleary and Glennie-Holmes (1985) using Megazyme International Ireland Ltd. (Bray, Ireland) enzymatic kit for β-glucan determination. Free glucose was calculated using blanks from β-glucan determination. Starch and pentosans were quantitated by the method of Holm et al (1986) and Hashimoto et al (1987), respectively. Protein was analyzed using a FP-428 Nitrogen Determinator (Leco Corp., St. Joseph, MI). Ash content was determined according to AACC Method 08-01 (AACC 1984). Lipid content was determined by refluxing samples with petroleum ether for 4 h using a Goldfisch apparatus according to AACC Method 30-25 (AACC, 1982). Four LAB extractions were combined into one large batch of BBG. All analyses were done at least in duplicate.

2.2.4. Viscosity measurements

Effect of shear

Pilot plant and LAB BBG solutions (0.5 and 1%, w/w) were prepared according to Burkus and Temelli (1998). Pseudoplastic behavior was described by the Power Law model (Eq. 2.1)

$$S = c\,R^n \tag{2.1}$$

where S is shear stress (N/m^2), R is shear rate (s^{-1}), c is consistency coefficient and n is flow behavior index. Viscosity was determined by consecutive fixed speed tests using a PAAR Physica UDS 200 rheometer (Glenn Allen, VA) equipped with a Peltier heating system. Tests were performed at 20±0.02°C using DG 27 cup and bob geometry with double gap and a 7 mL sample size. Sample size was not measured by volume due to the difficulty of transferring an exact sample volume. Instead, samples were weighed (7.01-±0.005 g) directly into the cup. Shear rate data are reported as rpm or as s^{-1} after multiplication by a conversion factor of 1.29 as specified by the manufacturer.

Stability tests

Viscosity tests for the determination of viscosity stability were carried out using a Haake Rotoviscometer (model RV-3, Gebruder Haake, Berlin, Germany) at 32 rpm. The viscometer was equipped with a MK 500 measuring head and NV viscosity sensor system (8 mL cup) with tempering vessel to maintain the temperature at 25±0.2°C.

Stability was determined as the change in viscosity over time (0, 1, 2, 6, 13 and 34 days). The laboratory solution was the BBG extract immediately before ethanol precipitation, which was taken as time=0. Extract was placed in a beaker and covered with Parafilm® and aluminum foil to prevent evaporation over time. Pilot Plant solution was prepared at 1% (w/w) initial concentration as described by Burkus and Temelli (1998), but the final concentration was not adjusted with distilled water to make up for evaporation losses in order to prevent microbial contamination. To prevent growth of microbial spores over the storage period, a common milk preservative, Brotab 10 (6 mg of Bronopol and 0.3 mg of Natamyicin per 20 mg tablet; Systems Plus, New Hamburg, ON), was added to solutions (1 tablet per 25 g solution).

2.3. RESULTS AND DISCUSSION

2.3.1. Yield, composition and viscosity of gums

The yield and composition of fractions obtained from lab and pilot plant extraction procedures are shown in Tables 2.1 and 2.2, respectively. The yield of PP gum was much

TABLE 2.1
Mass Balance of Fractions from 200 kg of Barley Flour (89.3% Solids) in Pilot Plant Extraction and from 200 g Flour (95.9% solids) in Laboratory Extraction of BBG Gums.

Fraction	**Amount kg**	**Solids %**	**Solids kg**	**β-Glucan kg**
Pilot plant extraction				
Starchy fraction 1 (HF 1)	288	35.1	101.1	3.0
Starchy fraction 2 (HF 2)	260	15.2	39.5	1.3
Protein-rich fraction (HF 3)	111	9.5	10.5	0.4
PP gum	7.05	94.7	6.7	5.6
Ethanol waste	3450	0.6	20.7	-
Total			178.5	10.3
Barley flour	200	89.3	178.6	12.8
LAB extraction	Amount, g		Solids, g	β-glucan, g
Starchy fraction	419	33.8	141.6	3.7
Protein-rich fraction	61.5	27.9	17.2	0.2
LAB gum	11.0	91.2	10.0	7.8
Ethanol waste	3340	0.6	20	-
Total			188.8	11.7
Barley flour	200	95.9	191.8	13.7

TABLE 2.2

Composition of Fractions Obtained in Pilot Plant and Lab Scale Extractions of β-Glucan Gum (% w/w, Dry Matter Basis)[a].

Fraction	Starch	Protein	Lipids	β-Glucan	Ash	Free Glucose
Barley flour	59.25±0.28	13.80±0.12	2.76±0.06	7.16±0.03	1.94±0.05	0.90±0.11
Pilot plant extraction						
Starchy fraction 1 (HF 1)	72.63 ±0.37	7.92±0.13	1.12±0.02	2.97±0.03	1.35±0.02	0.62±0.10
Starchy fraction 2 (HF 2)	59.69±0.03	23.22±0.07	3.02±0.11	3.30±0.11	2.52±0.04	0.47±0.02
Protein-rich fraction (HF 3)	1.56±0.14	56.61±0.13	27.47±0.15	3.34±0.14	3.64±0.01	0.23±0.03
PP gum	1.20±0.17	1.49±0.02	0	83.34±0.10	4.12±0.11	0
Lab extraction						
Starchy fraction	73.54±0.43	9.11±0.09	0.78±0.10	2.61±0.04	1.73±0.01	0.33±0.03
Protein-rich fraction	0.48±0.05	65.69±1.19	22.26±0.20	0.97±0.12	2.51±0.05	0.76±0.12
LAB gum	0.46±0.03	3.60±0.07	0	78.04±0.45	4.07±0.08	0

[a] mean (±SD)

lower than that of LAB gum, 3.5 vs. 5.5%. Several reasons, related to the operation of the pilot scale equipment, contribute to the lower yield achieved in the pilot plant. The PP extraction process lasted much longer than the anticipated one hour. The capacity of the first decanter for the separation of the HF1 fraction was about 500 kg/hr meaning that about 5 h was necessary for the complete separation of HF1 plus 2 h to establish the operation. Similarly, the time necessary for separation of HF2 was about 8 h. In reality, the extraction lasted an average 5.5 h. Then, the extract was supposed to be heated for 1 h at approximately 90°C in order to inactivate any surviving spores and enzymes. However, due to unanticipated problems with pH measurements under these conditions, the heat treatment actually lasted about 6 h. High pH is known to be potentially damaging for (1→3)-β-D bonds (McCleary and Codd 1991) and, at 90°C, some scission of (1→3) bonds probably started. As well, β-glucan may be susceptible to "peeling" reaction, which is the progressive erosion of monosaccharide units from the reducing end of the polysaccharide in alkaline conditions (White and Kennedy 1988). The loss of glucose units at, or close to the reducing end, and connected by (1→3) bond, which is more susceptible to peeling (White and Kennedy 1988), may partially explain the lower yield of PP gum. Random cleavage of β-glucan would produce shorter chain BBG with increased solubility in approximately 50% ethanol and would be simply washed away during β-glucan precipitation. Precipitated β-glucan also has shorter chains, which results in lower viscosity, as shown in Figure 2.2.

The relatively high β-glucan content of approximately 3% in the starchy fractions, HF1 and HF2 (Table 2.2), was probably caused more by the concentration effect due to drying, than by remnants of cell walls. This becomes more apparent if the β-glucan

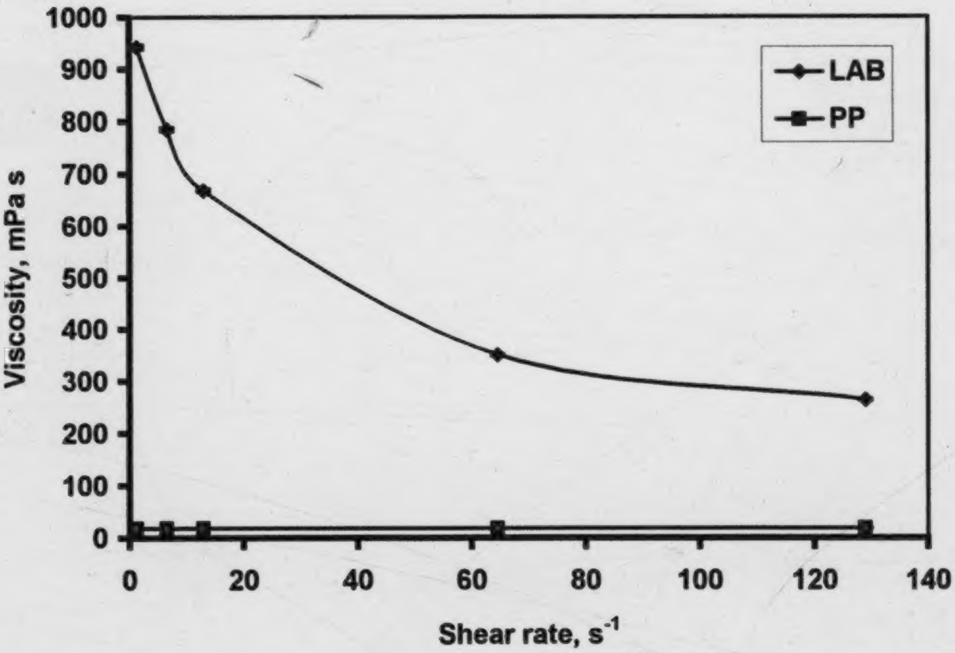

Figure 2.2. Effect of shear rate on viscosity of LAB and PP gums at 1% (w/w) concentration.

contents of protein-rich fractions obtained in pilot plant and laboratory are compared. The laboratory procedure uses higher centrifugal force and results in a pelleted precipitate with more solids than the HF3 fraction, which is in slurry form (Table 2.1). Such different mass distribution in separated fractions also caused increased losses of PP BBG gum.

The laboratory gum contained less β-glucan than did the PP gum (78.0% vs. 83.3%), which was partially offset by a higher pentosan content, 4.1% in LAB vs. 1.7% in PP gum. Pentosans are also soluble fiber components influencing viscosity (Bhatty et al 1991), and may be considered as a desirable impurity in BBG gum. The recovery of β-glucan, defined as the amount of β-glucan in the final gum as a percentage of the amount of β-glucan in the initial flour, was 43.5% and 57.0% for PP and LAB gum, respectively. Protein content was lower in PP gum (1.5% vs. 3.6%). The very long inactivation step probably denatured protein more completely. Protein sediment was still visible in a control sample tube after brief centrifugation and it is quite possible to achieve even higher purity by using a different centrifuge or filtration process at the pilot plant level. The higher starch content of PP gum was probably the consequence of the longer extraction procedure and increased content of damaged starch due to pin-milling. Such starch is more soluble at higher pH (Wurzburg 1972). Lower centrifugal force used at PP scale would allow for more of this "fluffy" starch to be transferred into the supernatant.

Both HF1 and starchy LAB fractions had increased starch content (72.6% and 73.5%, respectively) when compared with barley flour, which contained 59.3% starch. Both fractions contained less protein (7.9% and 9.1%, respectively) compared to 13.8% in the barley flour. These proteins are probably more of the hordein type, which are less soluble in water and aqueous alkali, but more soluble in alcohol (Shewry 1993). HF2 fraction resembles whole barley flour in composition except for its protein content (23.2% vs. 13.8%, respectively). This implies that it may be possible to further enrich certain barley fractions in starch and protein by a series of separation steps. Lower g-force would first separate a starch-rich fraction, while higher force would be necessary for the separation of a protein-rich fraction. These two fractions would be obviously targeted for further value added processing.

The acid precipitated fractions were very rich in protein (56.6% and 65.7% in fractions obtained in the pilot plant and lab, respectively) and lipid (27.5% and 22.3%, respectively). Oil was not visibly separated even after centrifugation at approximately 17,600×g in LAB extraction, which may indicate a very stable o/w emulsion. An oil content of >20% means that the protein-rich fraction may be a viable source of barley oil. Extraction of oil would further concentrate protein to >65% and >75% in pilot plant and lab fractions, respectively, which would then represent a true protein concentrate. Functional properties of such a concentrate are not known and may represent a challenge for future research.

Effect of shear on viscosity

The viscosities of PP and LAB gums were quite different, 17.9 vs. 668 mPa s at 10 rpm (Fig. 2.2). Laboratory gum was highly pseudoplastic with a flow behavior index n=0.71, while PP gum was almost Newtonian with n=0.99 in the 1-129 s^{-1} range (Table 2.3). Newtonian-like flow behavior of low viscosity (LV) BBG gums was already reported by Burkus (1996). The relatively high flow behavior index of LAB gum means that it has slippery mouthfeel and represents a potential fat replacer (Szczesniak and Farkas 1962). As well, it is easier to prepare solutions of LV gum in higher concentration (Burkus and

TABLE 2.3
Coefficients of Pseudoplasticity of 1% (w/w) PP and LAB Gum Solutions According to Power Law Equation (Data from Fig. 2.2)

Gum	Consistency Coefficient, c	Flow Behavior Index, n	R^2
LAB	1.186	0.713	0.9889
PP	0.018	0.997	1

Temelli 1999), which is necessary in some food systems. At low shear rates (1-20 s^{-1}), high viscosity (HV) LAB gum is quite comparable to the locust bean gum tested by Dawkins and Nnanna (1995).

There are several reasons for such a huge difference in viscosity between LAB and PP gums. Exposure to high pH for a long time is probably one of the main reasons as previously discussed. High shear during phase separation lowered viscosity of oat gum manufactured by Wood et al (1989) at the pilot plant level. For the production of PP gum, pilot scale industrial centrifuges were used five times for phase separation, while LAB gum was centrifuged in bottles in the absence of shear. However, a differently designed industrial process that uses less shear would probably be able to better preserve high viscosity of BBG gum. Product applications of HV and LV gums may be different, as discussed in Chapter 1, and each type may have advantages for specific food applications. However, the health benefits of PP and LAB gum may be different since health promoting effects of β-glucan seem to be related to viscosity (Wood 1994).

2.3.2. Stability of BBG gum

The viscosities of both PP and LAB gum solutions were stable for at least 13 days. The Laboratory gum solution exhibited stability for 34 days of testing, whereas PP gum lost about 28% of its original viscosity in 13-34 days period (Fig. 2.3). Neither gum solution showed any apparent signs of spoilage. Pilot Plant gum formed clumps of gel-like material upon standing for a month. Clumps were dispersed using a magnetic stirrer prior to viscosity measurements. This kind of retrogradation may limit the use of LV gums and applied concentrations must be <1% to slow down gelation and precipitation. The influence of the preservative used on PP gum gelation is not known and should be tested separately. When

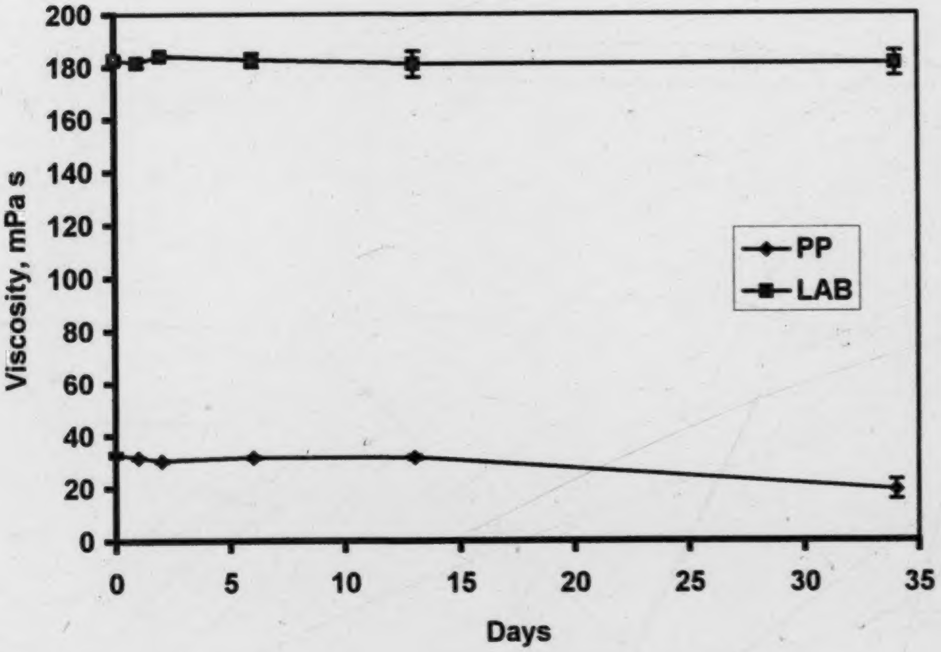

Figure 2.3. Viscosity stability of 1% (w/w) PP gum solution and fresh LAB extract as a function of time.

reheated, PP gum recovered part of its original viscosity, showing that its instability was predominantly of a physical nature.

2.4. CONCLUSIONS

High viscosity BBG gum can be extracted at high pH ($pH \approx 9$) and stabilized by applying heat at approximately 90°C for 1 h at pH 9. The Laboratory procedure, which had shorter holding times, resulted in a somewhat less pure gum (78.0% vs. 83.3% in PP gum) with a higher content of protein (3.6% vs. 1.5%) and pentosans (4.1% vs. 1.7%), but with a higher yield (5.5% vs. 3.5% for PP gum). The lower yield of PP gum was due to the higher moisture content of starch- and protein-rich residues and, probably, due to β-glucan cleavage by high shear and unanticipated exposure to high pH for a long time. Pilot-plant-extracted gum had viscosity of 17.9 mPa s vs. 668 mPa s for LAB gum (1% gum solution, w/w as is at 12.9 s^{-1}), which was the consequence of long holding times at elevated pH combined with high shear equipment. When HV gum is the target of extraction, the water:flour ratio should be increased to make protein precipitation more efficient.

Other fractions, such as the protein precipitate, look like promising materials for further utilization. Although extracted BBG gum was stable, further research is necessary to simplify the extraction procedure, increase β-glucan gum yield and preserve β-glucan viscosity during phase separation.

2.5. REFERENCES

AACC 1982. Approved Methods of the American Association of Cereal Chemists. American Association of Cereal Chemists, St. Paul, MN.

Bhatty, R. S. 1995. Laboratory and pilot plant extraction and purification of β-glucans from hull-less barley and oat bran. J. Cereal Sci. 22:163-170.

Bhatty, R. S., MacGregor, A. W., and Rossnagel, B. G. 1991. Total and acid soluble β-glucan content of hulless barley and its relationship to acid extract viscosity. Cereal Chem. 68:221-227.

Bhatty, R. S. 1999. The potential of hull-less barley. Cereal Chem.76:589-599.

Bhatty, R. S., and Rossnagel, B. G. 1997. Zero amylose lines of hull-less barley. Cereal Chem. 74:190-191.

Burkus, Z. 1996. Barley β-glucan: Extraction, Functional Properties and Interaction with Food Components. M. Sc. Thesis. University of Alberta, Edmonton, AB.

Burkus, Z., and Temelli, F. 1998. Effect of extraction conditions on yield, composition and viscosity stability of barley β-glucan gum. Cereal Chem. 75:805-809.

Burkus, Z., and Temelli, F. 1999. Gelation of barley β-glucan concentrate. J. Food Sci. 64:198-201.

Dawkins, N. L. and Nnanna, I. A. 1995. Studies on oat gum [(1→3, 1→4)-β-D-glucan]: composition, molecular weight estimation and rheological properties. Food Hydrocol. 9:1-7.

Hashimoto, S., Shogren, M. D., and Pomeranz, Y. 1987. Cereal pentosans: their estimation, and significance. I. Pentosans in wheat and milled wheat products. Cereal Chem. 64:30-34.

Holm, J., Bjorck, I., Drews, A., and Asp, N.-G. 1986. Rapid method for the analysis of starch. Starch/Starke 38:224-226.

Hurrel, R. F., and Finot, P. A. 1985. Effects of food processing on protein digestibility and amino acid availability. Pages 233-246 in: Digestibility and Amino Acid Availability in Cereals and Oilseeds. J. W. Finley and D. T. Hopkins, eds. AACC: St. Paul, MN.

McCleary, B. V. 1995. Personal communication. Megazyme International Ireland Ltd., Bray, Ireland.

McCleary, B. V. and Glennie-Holmes, M. 1985. Enzymic quantification of (1→3)(1→4)-β-D-glucan in barley and malt. J. Inst. Brew. 91:285-295.

McCleary, B. V. and Codd, R. 1991. Measurement of (1→3),(1→4)-β-D-glucan in barley and oats: a streamlined enzymic procedure. J. Sci. Food Agric. 55:303-312.

Morgan, K. R., and Ofman, D. J. 1998. Glucagel, a gelling β-glucan from barley. Cereal Chem. 75:879-881.

Shewry, P. R. 1993. Barley seed proteins. Pages 131-197 in: Barley: Chemistry and Technology. A. W. MacGregor and R. S. Bhatty, eds. American Association of Cereal Chemists: St. Paul, Minnesota.

Szczesniak, A. S., and Farkas, E. 1962. Objective characterization of the mouthfeel of gum solutions. J. Food Sci. 27:381-385.

White, C. A., and Kennedy, J. F. 1988. Identification and structural analysis of monomeric and polymeric carbohydrates. Pages 42-72 in Carbohydrate Chemistry. J. F. Kennedy, ed., Oxford University Press: New York, NY.

Wood, P. J. 1994. Evaluation of oat bran as a soluble fiber source. Characterization of of oat beta-glucan and its effects on glycaemic response. Carbohydr. Polym. 25: 331-336.

Wood, P. J., Siddiqui, I. R., and Paton, D. 1978. Extraction of high viscosity gums from oats. Cereal Chem. 55:1038-1049.

Wood, P. J., Weisz, J., Fedec, P., and Burrows, V. D. 1989. Large scale preparation and properties of oat fractions enriched in (1→3)(1→4)-β-D-glucan. Cereal Chem. 66:97-103.

Wurzburg, O. B. 1972. Starch in the food industry. Ch. 8 in: CRC Handbook of Food Additives. Second edition Vol. 1. T. E. Furia, ed. CRC Press, Inc.: Cleveland, OH.

Zheng, G. H., Han, H. L., and Bhatty, R. S. 1998. Physicochemical properties of zero amylose hull-less barley starch. Cereal Chem. 75:520-524.

3. DETERMINATION OF THE MOLECULAR WEIGHT OF BARLEY β-GLUCAN USING INTRINSIC VISCOSITY MEASUREMENTS[1]

3.1. INTRODUCTION

Molecular weight (MW) is one of the most fundamental parameters characterizing a macromolecule (Varum et al 1992). MW is related to solution properties of polysaccharides such as intrinsic viscosity at low concentrations and flow behavior at higher concentrations (Robinson et al 1982). Data on molecular weight of β-glucan are variable (Dawkins and Nnanna 1995), and to a great extent depend on the testing method and MW standards used for calibration.

Molecular weight of barley and oat β-glucan (BBG and OBG, respectively) can be determined by different techniques. Wood et al (1989) used high performance gel chromatography to determine the MW of oat β-glucan. The column was calibrated with pullulan standards. Later, Wood et al (1991) found that the MW standards should also be OBG since the use of pullulan led to significant overestimation of OBG MW. The problem is to properly measure MW standards. Morris (1989) stressed the need to determine the parameters in the Mark-Houwink relationship by calibration against an absolute method of MW measurement such as light scattering. Using light scattering, Varum et al (1992) determined the average MW of OBG and concluded that approximately 10% of OBG was in the form of reversible aggregates, which were largely dissociated at concentrations <0.2 g/L.

Another method of MW determination is through the measurement of intrinsic viscosity [η]. Intrinsic viscosity is independent of concentration (c) by virtue of extrapolation of reduced viscosity to c=0 and usually is related to molecular weight

---

[1] A version of this chapter is to be submitted to Carbohydrate Polymers for consideration for publication.

(Boucher and Alves 1973). Grimm et al (1995) determined the intrinsic viscosity of BBG from beer at 20°C and at a shear rate of 30 s^{-1}. They found that beer BBG completely dissociated in cuoxam (copper (II) tetramine-hydroxide) giving an approximate MW of 175,000. Because of a relatively small increase in gyration radius of aggregates compared to MW growth, they proposed a fringed micelle type of β-glucan aggregation with a degree of association >17 for short beer β-glucan, while longer OBG analysed by Varum et al (1992) had a degree of association 4-5. Similarly, reversible dissociation of (1→3)-β-D-glucan from *Poria cocos* sclerotium in cadoxen (saturated CdO solution in 29% ethylenediamine) was described by Zhang et al (1997). They speculated that the regularity of structure may be responsible for aggregation of this type of β-glucan.

Intrinsic viscosity depends on solvent type. The presence of sugars generally increases apparent viscosity while decreasing intrinsic viscosity (Elfak et al 1977). Grimm et al (1995) tested the influence of maltose on beer β-glucan and reported minimum [η] at a maltose concentration of 5%. A drop in temperature increased both apparent and intrinsic viscosity of beer β-glucan while the presence of 5% ethanol increased intrinsic viscosity and enhanced precipitation (Linemann and Kruger, 1998a).

Intrinsic viscosity may also be used to calculate critical concentration, c*, which is the concentration at which molecular entanglement begins. For most hydrocolloids, it is calculated according to the formula $c^* \approx 4/[\eta]$ (Morris 1989), with the exception of galactomannans, guar and locust bean gum, where $c^* \approx 2.5/[\eta]$. In all cases, viscosity at c* is about 10 mPa s. Gum concentrations above c* may suppress the taste and flavor of the product the gum is incorporated into due to an increase in viscosity (Morris 1989).

Determination of [η] and c* would contribute to an understanding of BBG behavior in a solution or more complicated food systems and perhaps lead to successful product applications. The goal of this study was to determine the MW and c* of pilot plant (PP) and laboratory obtained (LAB) BBG gums from intrinsic viscosity measurements using BBG MW standards.

3.2. MATERIALS AND METHODS

3.2.1. Materials

Pure BBG MW standards (32,000, 143,000, 212,000, 327,000 and 443,000) were obtained from Megazyme International Ireland Ltd. (Bray, Ireland). MW, as specified by the supplier, was determined by multi angle laser light scattering in NaOH as solvent. Pilot Plant and LAB gums were obtained as described in Chapter 2. Distilled water was from the local supply.

3.2.2. Preparation of β-glucan solutions

Stock solutions for intrinsic viscosity determination were prepared in duplicate at a concentration of 0.2% (w/w). A dry 50 mL beaker was weighed and tared, water was added in the amount required for stock solution preparation, and the beaker with water and a magnetic stirring bar was weighed precisely to ±1 mg. The beaker was emptied and dried with compressed air. BBG was weighed precisely into the beaker and the magnetic stir-bar was added. The required amount of water to achieve the desired concentration was then added simultaneously with the beginning of stirring. After 5 s, the beaker was transferred onto a preheated hot plate, covered with aluminum foil and quickly brought to a boil while stirring. Heating was continued for 1 h at 85°C in a water bath. After cooling to room temperature, the beaker was wiped carefully to remove excess condensation and the weight was adjusted to cover for evaporative losses. The beaker was immediately covered with Parafilm® (American Can Company, Greenwich, CT) and aluminum foil and stirred for an additional 30 s. Each stock solution was further diluted into duplicate lines of samples having concentrations of 0.100%, 0.075%, 0.050% and 0.025% (w/w). The concentration was adjusted by weighing the necessary amounts of sample and adding distilled water into capped vials on the balance (i.e. 4.000 g of β-glucan solution + 4.000 g of distilled water). Capped vials were previously pasteurized by wet heat to prevent any microbial growth during measurements. Diluted samples were vigorously stirred on a vortex mixer and left to equilibrate at least 15 min prior to viscosity measurements.

3.2.3. Viscosity measurements

Viscosity was determined by a fixed speed test at 20 rpm (25.8 s^{-1}) using a PAAR Physica UDS 200 rheometer (Glenn Allen, VA) equipped with a Peltier heating system. The instrument was calibrated with S3 standard oil (3.89 mPa s at 20°C, Cannon Instrument Co., State College, PA) for low viscosity (LV) measurements and Brookfield 500 cps standard oil (482 mPa s at 25°C, Brookfield Engineering Laboratories, Inc., Middleboro, MA) for high viscosity (HV) measurements. Temperature was calibrated with a thermocouple. Tests were performed at 20°C±0.02°C using the DG 27 cup and bob geometry with double gap and 7 mL sample size. Sample size was not measured by volume but by weight. The clean DG 27 cup was placed on the balance, tared, and 7.000 ± 0.005 g of sample was measured directly into the cup.

Viscosity was measured 10 times in one run for each sample, the rheometer was stopped and started anew 10 times so that each viscosity value used for the calculation of reduced viscosity represented an average of 100 measurements (i.e. ten measurements for each of ten runs).

3.2.4. Determination of intrinsic viscosity, molecular weight and critical concentration

Reduced viscosity was calculated as described by Linemann and Kruger (1998b):

$$\eta_{red} = (\eta - \eta_0) / \eta_0 \, c \qquad (3.1)$$

where η is the sample viscosity, η_0 is the viscosity of the solvent (distilled water) and c is the concentration of pure hydrocolloid (g/mL). The concentration of standard barley β-glucan gums was corrected according to manufacturers specifications of 7% moisture and 99.5% purity. The presence of pentosans in BBG gum also contributes to viscosity (Bhatty et al 1991). Therefore, for PP and LAB gums, the total concentration of pure β-glucan (78.92% and 71.14%, as is basis, respectively) and pentosans (1.64 and 3.75% as is, respectively) was used in calculations as 80.6 and 74.9% as is, respectively.

Intrinsic viscosity [η] was calculated by linearly extrapolating the reduced viscosity to zero concentration from concentrations of 0.025%-0.1%, or exponentially from concentrations of 0.025%-0.2%. Linearly extrapolated [η] was used to determine the relative MW of LAB and PP gums from the linear plot of MW vs. [η]. Exponentially determined [η] was further used in the Mark-Houwink relationship (Eq. 3.2) to determine BBG relative MW and BBG solution behavior.

$$[\eta] = K' \, M_r^{\alpha} \qquad (3.2)$$

where M_r is relative molecular weight and K' and α are "stiffness" parameters calculated from a double logarithmic plot of [η] against M_r.

Critical concentration (c*) was first determined as

$$c^* \approx 4/[\eta] \qquad (3.3)$$

as described by Robinson et al. (1982), and then as

$$c^* \approx 2.5/[\eta] \tag{3.4}$$

as suggested by Doublier and Wood (1995) for high viscosity OBG.

3.3. RESULTS AND DISCUSSION

3.3.1. Intrinsic viscosity and molecular weight

The viscosities of β-glucan MW standards and PP and LAB gums in the concentration range 0.25-1% (w/w) are depicted in Figure 3.1. Laboratory gum and the 443K standard had very similar viscosities at different concentrations (at 20 rpm), but, due to its lower purity, the reduced viscosity of LAB gum was much higher (Fig. 3.2). The highly viscous 443K gum exhibited an exponential increase in viscosity, even at a concentration of 0.1%, which was also reflected in the non-linearity of reduced viscosity (Fig. 3.2). The reduced viscosities of the other gums were quite linear below 0.1% concentration. Their intrinsic viscosities at c→0, as determined by linear extrapolation, are shown in Table 3.1. The intrinsic viscosities of MW standards were plotted in a linear standard curve (Fig. 3.3) from which the relative MW of PP and LAB gums were determined to be 198K and 598K, respectively.

It is clearly visible from Figure 3.3 that intrinsic viscosity will reach 0 before MW drops to zero, which is logical since the viscosity of oligosaccharide solutions at low concentrations will be close to that of water. The surprise was the complete linearity of the plot with excellent R^2 (0.9985). Robinson et al (1982) also obtained excellent linearity of intrinsic viscosity for guar gum (R^2>0.99), but the intercept [η] on the y axis when

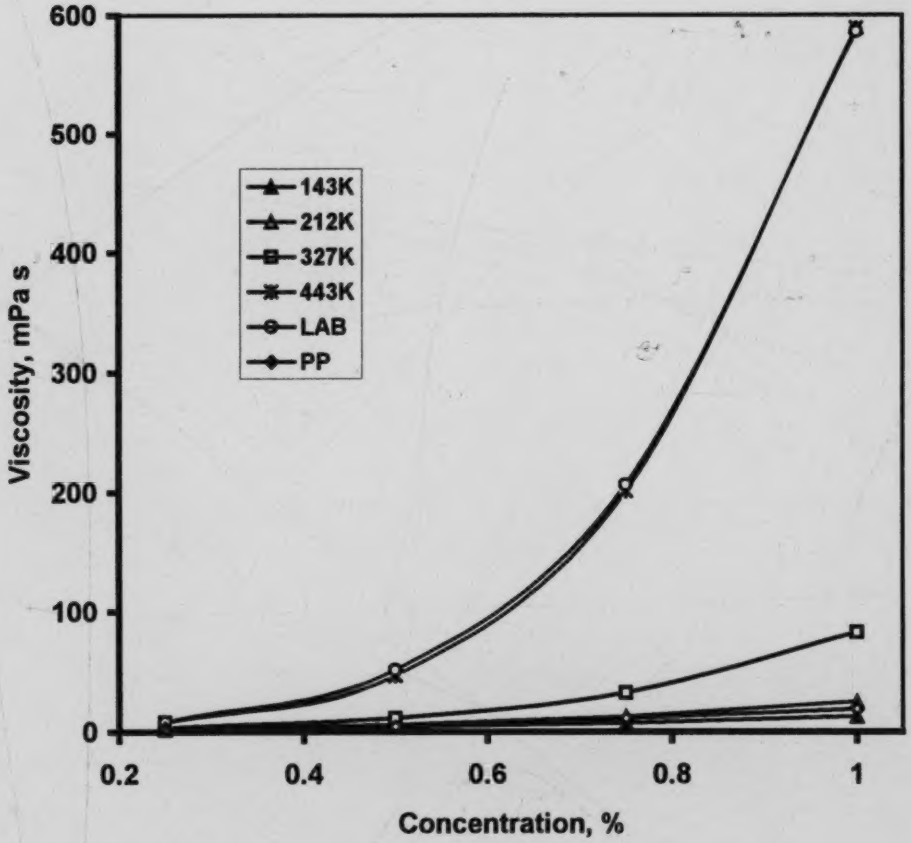

Figure 3.1. Viscosity of MW standards, PP and LAB gums in the concentration range 0.25-1% (w/w). The 32K standard was not included due to its very low viscosity (<2 mPa s).

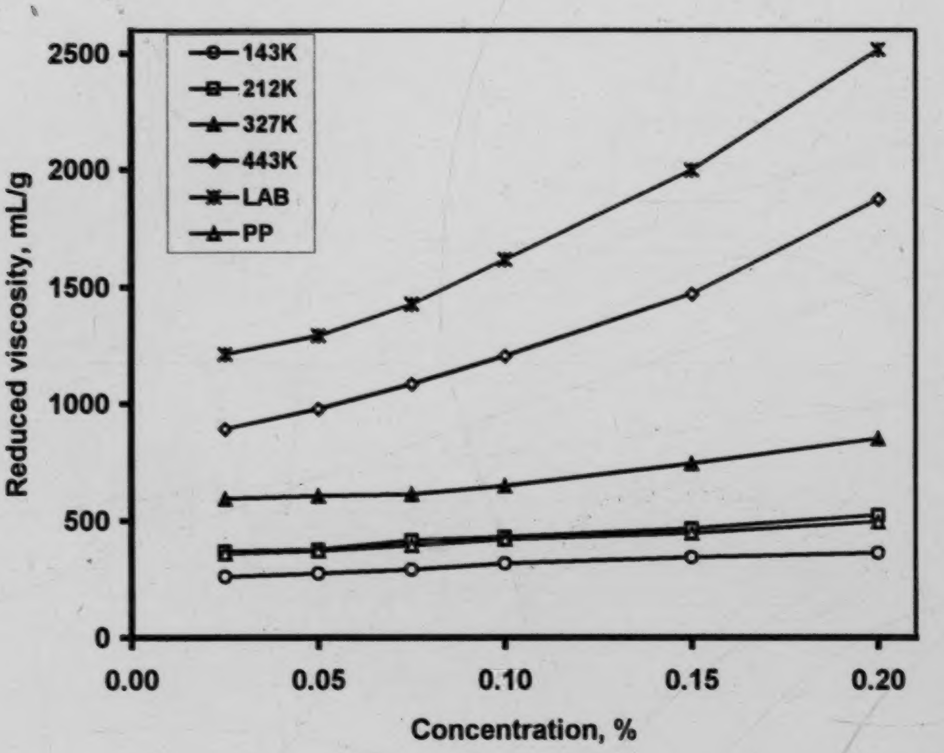

Figure 3.2. Reduced viscosities of BBG MW standards, PP and LAB gum in the concentration range 0.025-0.2% (w/w). 32K not shown.

TABLE 3.1

Intrinsic Viscosity [η] of MW Standards, PP and LAB Gums After Linear Extrapolation from ≤0.1% Concentration to c→0, or Polynomial Extrapolation from 0.2% to c→0.

Gum	Linear [η], mL/g	Polynomial [η], mL/g
32K	44	44[1]
143K	238	238[1]
212K	339	347
327K	570	588
443K	776	835
LAB	1049	1125
PP	333	338

[1] The same as linear for MW calculations; polynomial extrapolation not applicable.

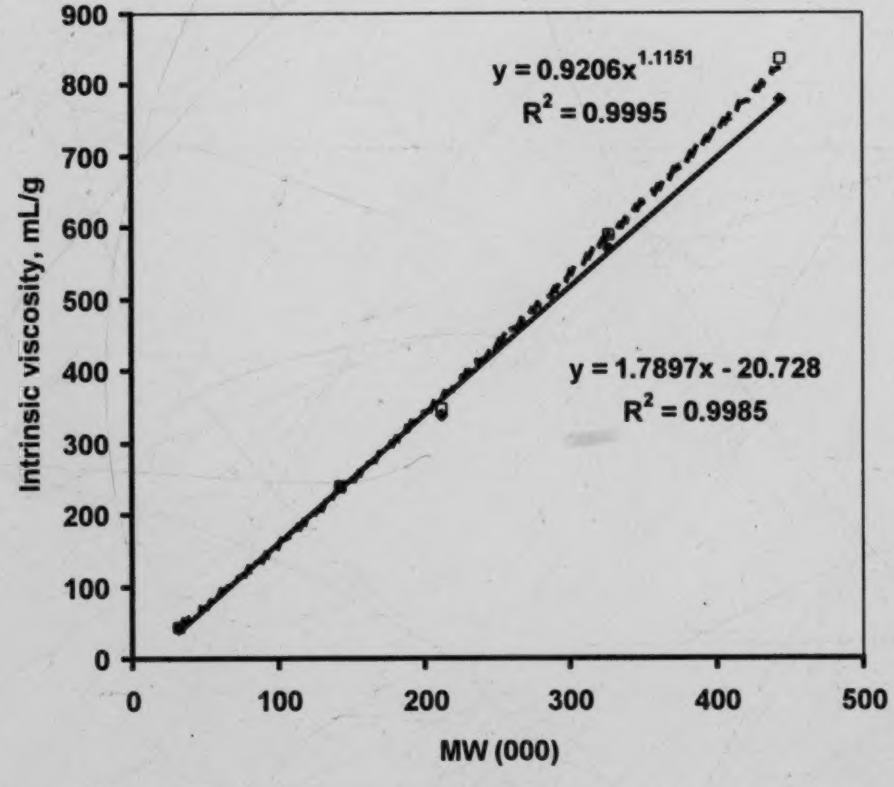

Figure 3.3. Intrinsic viscosity standard curve determined linearly (solid line) from the linear extrapolation of [η] to c→0 (solid symbols), or determined from Mark-Houwink relationship (dotted line) from exponential extrapolation of [η] to c→0 (open symbols).

MW→0 was 151 mL/g, which is impossible. After fitting their data to the Mark-Houwink relationship (Eq. 3.2), they obtained $\alpha=0.723$, while such a fitting in this study resulted in $\alpha=1.09$. This finding is much different from Grimm et al (1995) who determined $a=0.725$ ($a = \alpha$) for beer β-glucan. In their case, the same β-glucan was tested in different solvents and the exponent 'a' represented aggregation behavior of beer β-glucan. This exponent was used by Linemann and Kruger (1998b) to determine the MW of their β-glucan.

However, this calculation should not be confused with the Mark-Houwink relationship, which depicts the type of conformation assumed by the polymer in solution. The value of $\alpha=1.09$ is the lowest possible number calculated in this study. If $[\eta]$ for more viscous 327K and 443K samples is calculated based on the three lowest concentrations (0.025-0.075%), α increases to 1.10, while calculation of $[\eta]$ for 443K, 327K and 212K using the polynomial extrapolation (Table 3.1) results in $\alpha=1.115$. This finding implies that barley β-glucan behaves like a partially stiffened coil or wormlike chain (Robinson et al 1982, Gomez et al 1997). Using this higher α value in Eq. 3.2 for MW estimation results in a relative MW for PP gum of 199K, which is similar to the estimate of 198K based on linear extrapolation. Extrapolation of the Mark-Houwink equation for LAB gum results in a MW estimate of 585K, or about 2% less than that obtained from the linear relationship of MW vs. $[\eta]$. Therefore, the linear standard curve can be applied to the estimation of BBG MW in the range 40K-600K. For higher MW, the Mark-Houwink relationship should be used, but even that may be simplified with higher MW standards.

The coefficient $a=0.725$ in Grimm et al (1995) for beer β-glucan actually shows a decreasing trend as viscosity increases with MW, meaning that at concentrations above the critical value c*, β-glucan micelles grow more laterally, slowly increasing in diameter. For lower viscosity β-glucan with shorter chain length, this coefficient may be even lower due to pronounced lateral aggregation. It was noticed in this study that the 32K sample at 1% concentration had roughly the same reduced viscosity as at 0.75% concentration. This measurement was repeated with another similar sample having a MW of 31K. Both samples had viscosity <2 mPa s at 1% concentration (w/w).

Extrapolation of the linear standard curve in Figure 3.3 gives an intercept for $[\eta]=0$ at MW=11.58K, which corresponds to approximately 71 glucose units in a polymer. However, if the data for only the three lower MW standards were used, an intercept on the MW axis at 3.38K is obtained, which is approximately 21 glucose units. This would be the point when BBG loses solubility because of very low viscosity and enhanced aggregation into an insoluble precipitate. This is in agreement with Doublier and Wood (1995) who found that, after digestion with lichenase, OBG yielded an insoluble precipitate composed mostly of glucose oligosaccharides with 9-15 glucose units.

3.3.2. Critical concentration

The critical concentration c* for PP and LAB gums was determined (Eq. 3.3) to be 12 and 3.8 g/L, respectively, or 1.2 and 0.38% of pure β-glucan, or 1.49% and 0.51% of gum. If the value of $[\eta]=1125$ mL/g for LAB gum, obtained after polynomial extrapolation of reduced viscosities, is used for c* calculation, then $c^*\approx0.36\%$ for pure β-glucan. Both gums should have a viscosity of about 50 mPa s (or slightly higher) at c*.

Applying the same formula, $c^* \approx 4/[\eta]$, to HV 327K β-glucan yields $c^*\approx0.76\%$. The viscosity of this gum at a similar concentration of 0.75% was 32.8 mPa s, which was sharply increased from 11 mPa s at 0.5% concentration. This finding indicates that the critical concentration was already achieved. Doublier and Wood (1995) distinguished first and second entanglement points in the behavior of OBG corresponding to $c^*[\eta]=0.7$ and $c^*[\eta]= 2.5$, respectively. Using equation $c^*[\eta] \approx 0.7$ results in $c^* \approx 0.13\%$ for 327K β-glucan with a viscosity of <2 mPa s. It is hard to believe that such a low viscosity would affect taste perception during sensory evaluation. Application of the second entanglement point of $c^*[\eta] \approx 2.5$ results in critical concentration at 0.47% with viscosity estimated between 10-11 mPa s, which is in the expected region. The same calculation for LAB gum results in $c^* \approx 0.30\text{-}0.32\%$, which probably yields viscosity in the vicinity of 10 mPa s since a 0.25% solution had viscosity of 7.7 mPa s.

Pilot Plant gum at ≥1% concentration displays time dependent behavior e.g. a gelling tendency stimulated by low shear rates, as will be discussed in Chapters 4 and 5. Therefore, any conclusions about determination of c* from Eq. 3.3, as proposed by Robinson et al (1982), may be premature for LV BBG gums. The calculation of c* for PP gum from Eq. 3.4 resulted in $c^* \approx 0.93\%$, with an expected viscosity of about 17 mPa s. Pilot Plant gum at 0.93% concentration is still prone to gelling over time, as will be discussed in Chapters 5 and 6. Due to the formation of a network, its solution actually becomes a weak gel with a yield point and much higher viscosity. Applying the same equation (Eq. 3.4) to 143K β-glucan results in $c^* \approx 1.14\%$. At that concentration, 143K gum would gel even faster than PP gum because of its lower viscosity. Therefore, using the equation $c^*[\eta] \approx 2$ may give better results for low viscosity, gel forming BBG. However, it is questionable how PP gum behaves at 0.74% concentration and proper determination of critical concentration for this type of BBG gum may require additional experimental measurements. Application of even this lowered c* estimate on 32K gum yields c*≈4.9%. At that concentration, this very LV BBG gum will probably gel in a matter of hours, if not minutes. Therefore, determination of c* for gel-forming LV BBG is probably out of the question. Above c*, flavor and taste release are retarded for almost all food hydrocolloids (Morris, 1989). This is probably true for fresh β-glucan gum solutions as well.

3.4. CONCLUSIONS

The intrinsic viscosity of BBG MW standards follows a linear relationship of MW vs. [η] with R^2>0.99%, based on linear extrapolation of reduced viscosity at concentrations ≤0.1%. The relative MW of PP and LAB gums was determined to be 198,000 and 598,000, respectively. Low molecular weight β-glucan (32K) exerts stagnation in reduced viscosity rise at concentrations above 0.75%, probably due to lateral aggregation. Critical concentration c* for PP and LAB gums was calculated from c* ≈ 4/[η] to be approximately 1.49% and 0.51% of gum, respectively. Viscosity at critical concentration would be about 50 mPa s, higher than the commonly accepted value of about 10 mPa s. The equation c*[η]≈2.5 seems to be suitable for the calculation of critical concentration for HV BBG, as already determined by Doublier and Wood (1995) for OBG, while determination of c* for LV BBG probably requires experimental confirmation of applied formulas. Time-dependent aggregation-gelation of PP gum and, generally, LV BBG gums, may void any practical application of c* in certain types of products.

3.5. REFERENCES

Bhatty, R. S., MacGregor, A. W., and Rossnagel, B. G. 1991. Total and acid soluble β-glucan content of hulless barley and its relationship to acid extract viscosity. Cereal Chem. 68:221-227.

Boucher, D. F., and Alves, G. E. 1973. Introduction to fluid mechanics. Pages 5-3 - 5-61 in: Chemical Engineers Handbook. R. H. Perry and C. H. Hilton, eds., McGraw-Hill Kogakusha, Ltd.: Tokyo, Japan.

Dawkins, N. L. and Nnanna, I. A. 1995. Studies on oat gum [(1→3, 1→4)-β-D-glucan]: composition, molecular weight estimation and rheological properties. Food Hydrocol. 9:1-7.

Doublier, J. L., and Wood, P. J. 1995. Rheological properties of aqueous solutions of (1→3) (1→4)-β-D-glucan from oats (*Avena sativa* L.). Cereal Chem. 72:335-340.

Elfak, A.M., Pass, G., Philips, G.O., and Morley, R.G. 1977. The viscosity of dilute solutions of guar gum and locust bean gum with and without added sugars. J. Sci. Food Agric. 28: 895-899.

Gomez, C., Navarro, A., Manzanares, P., Horta, A., and Carbonell, J.V. 1997. Physical and structural properties of barley (1→3),(1→4)-β-D-glucan II. Viscosity, chain stiffness and macromolecular dimensions. Carbohydr. Polym. 32:17-22.

Grimm, A., Kruger, E., and Burchard W. 1995. Solution properties of β-D-(1,3)(1,4)-glucan isolated from beer. Carbohydr. Polym. 27:205-214.

Linemann, A., and Kruger, E. 1998a. Relationships between structure and characteristics of β-glucan in beer production. Part 2: Changes in state of dissolution of β-glucan and formation of β-glucan gel during the brewing process. Brauwelt Int. 1998(II):132-139.

Linemann, A., and Kruger, E. 1998b. Relationships between structure and characteristics of β-glucan in beer production. Part 3: Determination of limited viscosity number of β-glucan in worts-A method for predicting filterability on β-glucan side. Brauwelt Int. 1998(III): 214-220.

Morris, E.R. 1989. Polysaccharide solution properties: origin, rheological characterization and implications for food systems. Pages 132-163 in: Frontiers in Carbohydrate Research-1: Food Applications. R.P. Millane, J.N. BeMiller, and R. Chandasekaran (eds) Elsevier Applied Science: New York, N. Y.

Robinson, G., Ross-Murphy, S.B., and Morris, E. R. 1982. Viscosity-molecular weight relationships, intrinsic chain flexibility, and dynamic solution properties of guar galactomannan. Carbohydr. Res. 107:17-32.

Varum, K. M., Smidsrod, O., and Brant, D. A. 1992. Light scattering reveals micelle-like aggregation in the (1→3),(1→4)-β-D-glucan from oat aleurone. Food Hydrocol. 5:497-511.

Wood, P. J., Weisz, J., Fedec, P., and Burrows, V. D. 1989. Large scale preparation and properties of oat fractions enriched in (1→3)(1→4)-β-D-glucan. Cereal. Chem. 66:97-103.

Wood, P. J., Weisz, J., and Mahn, W. 1991. Molecular characterization of cereal β-glucans II, size exclusion chromatography for comparison of molecular weight. Cereal Chem. 68:530-536.

Zhang, L., Ding, Q., Zhang, P., Zhu, R., and Zhou, Y. 1997. Molecular weight and aggregation behavior in solution of β-D-glucan from *Poria cocos* sclerotium. Carbohydr. Res. 303:193-197.

4. RHEOLOGICAL PROPERTIES OF BARLEY β-GLUCAN[1]

4.1. INTRODUCTION

Barley and oat β-glucan (BBG and OBG, respectively) show similarities in structure both having about 90% cellotriosyl and cellotetraosyl units. Oat β-glucan usually exerts somewhat higher viscosity due to longer molecular chains (Wood et al 1991, Beer et al 1997), but it may be expected that at similar molecular weights both β-glucans behave alike. Thus, the following review of β-glucan rheological properties includes both gums.

The pseudoplasticity of β-glucan gums is already an established fact with high viscosity (HV) gums having a high consistency coefficient and low (<<1) flow behavior index of the Power Law equation (Eq. 2.1). Bhatty (1995) and Burkus (1996) reported a flow behavior index >0.7 for both BBG and OBG gums, as was confirmed in Chapter 2 of this thesis. Flow behavior index values closer to one implies good fat replacing properties (Szczesniak and Farkas 1962). However, Autio et al (1987) found that very high viscosity oat gum at concentrations ≥1% (w/w) had a flow behavior index <0.3, which means that the recommended concentration of such gums for potential fat replacement is ≤0.5%. Low viscosity (LV) gums and HV gums at low concentrations behave like Newtonian fluids (Burkus 1996). The concentration of HV gums at which they exhibit pseudoplasticity may be as low as 0.2% (Autio et al 1987).

Temperature causes reversible changes in the viscosity of β-glucan gums (Dawkins and Nnanna 1995). Generally, viscosity decreases with temperature, but the expected viscosity decrease may show some deviation for BBG with increasing temperature. For example, Dawkins and Nnanna (1995) found that the viscosity of OBG gum (0.5%, w/w) increased with temperature from 25-37°C, while viscosity at 61°C was slightly below that of the control at 25°C. At 100°C, viscosity was much lower than that at 25°C. Gomez et al (1997) determined the viscosity of 1.5% BBG solution at low shear rates (<3 s^{-1}) and 70°C to be higher than that at 25°C. While their conclusion about β-glucan aggregation at high temperatures is somewhat questionable (as discussed in Chapter 1), some viscosity deviation due to structural changes of β-glucan micelles is quite possible. Therefore, there is a need for further elucidation of BBG behavior at elevated temperatures, especially under conditions that may have industrial processing implications.

---

[1] A version of this chapter is to be submitted to Food Hydrocolloids for consideration for publication.

Thixotropy is the decrease in viscosity under constant shear rate over a period of time. Thixotropy of BBG from beer was investigated by Linemann and Kruger (1997). The procedure applied was flow curve determination where shear rate was increased from 0 to 120 s^{-1} and then decreased back to 0 s^{-1} in 8 min. They found that thixotropy disappeared at 55°C, which could be caused by melting of the micelle structure (Morgan and Ofman 1998, Burkus and Temelli 1999) and a drop in viscosity due to increased temperature. Conversely, Autio et al (1987, 1992), Wood et al (1989) and Wikstrom et al (1994) did not observe thixotropy in the behavior of OBG gum. While quick gelation of the hydrolyzed beer BBG in the samples of Linemann and Kruger (1997) and dispersion of gel particles upon shearing cannot be completely precluded as a source of thixotropy, inertia of the measurement system may also contribute to such behavior. Inappropriateness of flow curve measurements for viscosity tests was already demonstrated by Burkus (1996) and the only relevant test would be a step-wise test, which is in agreement with Speers (1999).

Storage modulus (G') and loss modulus (G'') are variables used by Doublier and Wood (1995) to demonstrate gel-like behavior of partially hydrolyzed oat gum. Gelation of LV β-glucan was confirmed by Burkus and Temelli (1999) and Morgan and Ofman (1998). Gelling ability means the formation of a three-dimensional network that sets to form a solid-like product trapping the water within the network. The existence of a network in a hydrocolloid solution results in a yield point. However, determination of the yield point is very difficult when a hydrocolloid is present at a low concentration because the measurement of a very low shear stress is necessary. Increasing gum concentration may not help in yield point determination since it enhances gelling and shortens the setting time (Burkus and Temelli 1999). The creation of a network by 2% (w/w) wheat gum solution composed of β-glucan and pentosans (83.5 % purity, 77% pentosans, 22.9% β-glucan) was reported by Cui et al (1999) when they measured G' and G'' development over time. It has been observed (data not published) that LV β-glucan solutions and emulsions gelled after long holding periods even at concentrations ≤1%, with a gel settling out of aqueous solution. This observation indicates that a network is first formed, but the time dependence of gelation behavior is not known.

Therefore, the objectives of this study were:

- to investigate the effects of temperature and shear rate on the viscosity of laboratory (LAB) and pilot plant (PP) extracted BBG gums and to use the Arrhenius and Power Law equations to describe their respective effects,
- to verify thixotropic behavior of BBG gums,
- to study network formation in BBG solutions through monitoring changes in G' and G'' over time, and

- to test the existence of yield point in BBG solutions.

4.2. MATERIALS AND METHODS

4.2.1. Materials

Pilot Plant and LAB BBG gums were extracted from waxy barley as previously described in Chapter 2. Additional LV BBG was extracted from Condor barley (a regular starch type), as described in Burkus and Temelli (1999). β-Glucan solutions were prepared in the desired concentration (w/w) according to by Burkus and Temelli (1998). Distilled water was from the local supply.

4.2.2. Rheological measurements

Viscosity was determined by consecutive fixed speed tests using a PAAR Physica UDS 200 rheometer (Glenn Allen, VA) equipped with a Peltier heating system. The instrument was calibrated with S3 standard oil (3.408 mPa s at 25°C, Cannon Instrument Co., State College, PA) for LV measurements and Brookfield Viscosity Standard - Fluid 500 standard oil (482 mPa s at 25°C, Brookfield Engineering Laboratories, Inc., Middleboro, MA) for HV measurements. Tests were performed at the desired temperature (±0.03°C) using the DG 27 cup and bob geometry with double gap and a 7 mL sample size. Sample size was not measured by volume but by weight. The clean DG 27 cup was placed on the balance, tared and 7.01 ± 0.005 g of sample was weighed directly into the cup. Preset variables such as time, temperature, shear rate or speed, the number of measurement points and raw values per point, which were entered into the Measurement window of US200-V.2.04 software, are shown in Table 4.1. Shear rate data are reported as rpm or, after multiplication by the conversion factor of 1.29, as s^{-1}.

TABLE 4.1

Parameters in the Measurement Window for the Measurement of PP and LAB Gum Thixotropy at 1-3000 rpm and 20°C.

	INTERVAL								
	1	2	3	4	5	6	7	8	9
LAB 1000 rpm									
Rotation, rpm	1	5	10	20	50	100	1000	1	-
Measuring points, #	7	7	7	7	7	7	20	8	-
Meas. point duration	1 min	12 s	6 s	3 s	3 s	3 s	3 s	1 min	-
Raw values/point	20,000	4,000	2,000	1,000	Automatic	Automatic	Automatic	20,000	-
LAB 3000 rpm									
Rotation, rpm	1	5	10	20	50	100	1000	3000	1
Measuring points, #	4	7	7	7	7	7	7	20	10
Meas. point duration	1 min	12 s	6 s	3 s	3 s	3 s	3 s	3 s	1 min
Raw values/point	20,000	4,000	2,000	1,000	Automatic	Automatic	Automatic	Automatic	20,000
PP 1000 rpm									
Rotation, rpm	5	10	20	50	100	1000	5	-	-
Measuring points, #	7	7	7	7	7	20	10	-	-
Meas. Point duration	12 s	6 s	3 s	3 s	3 s	3 s	12 s	-	-
Raw values/point	4,000	2,000	1,000	Automatic	Automatic	Automatic	4,000	-	-

Pseudoplastic behavior was described by the Power Law model (Eq. 4.1):

$$S = c\,R^{n} \qquad (4.1)$$

where S is shear stress (N/m^2), R is shear rate (s^{-1}), c is the consistency coefficient and n is the flow behavior index.

The viscosity of 1% (w/w) LAB and PP gum solutions was determined at temperatures ranging from 0.1°C to 75°C, in duplicate. The effect of temperature on viscosity is described by the Arrhenius equation:

$$\eta_a = A\, e^{(-E_a/RT)} \qquad (4.2)$$

where η_a is apparent viscosity, A is a constant, E_a is activation energy (J/mol), R is the gas constant (8.314 J/mol K) and T is temperature (°K). After logarithmic transformation, Equation 4.2 becomes a linear equation:

$$\ln \eta_a = \ln A - (E_a/R)\,(1/T) \qquad (4.3)$$

where E_a/R represents the slope of the line obtained by plotting ln η_a as a function of 1/T. Since E_a has been reported to be both concentration and shear dependent (Autio et al 1987), E_a was determined over the shear rate range 1.29-25.8 s^{-1}.

Storage and loss modulus (G' and G'', respectively) were recorded in overnight tests of at least 18 h duration. The UDS 200 rheometer was in amplitude sweep - controlled shear displacement (CSD) mode with a constant strain of 3-5% (γ in the measurement window) and frequency of 0.5-1 Hz. Data points were recorded every 5-10 min. All samples were prepared in duplicate; PP and Condor gums were tested at 1%, 2.5% and 5% (w/w) concentration, whereas LAB gum was tested at 1% concentration. To prevent evaporation and formation of a skin-like layer on the surface of the samples during overnight tests, 0.5 mL of S3 standard oil was layered onto the samples in the

DG27 cup. Samples were placed in the cup and the bob was brought into measurement position, and slowly rotated by hand to wet the walls of the cup and bob uniformly. Then, the bob was pulled out 5-6 mm and approximately one half of the oil was dosed onto the sample inside the concentric cylinder of the bob. The bob was placed back into measurement position, the remainder of the oil was put into the outside gap, and the test was started.

The Yield point of the created network was tested using the same amplitude sweep -CSD testing mode with strain increasing 2-100% during automatic flow curve measurement at a frequency (ω) of 1 Hz, with 30-50 points recorded. There is no time setting for this kind of test. Condor and PP gum solutions at 2.5% and 5% concentration were tested in duplicate, before and after overnight testing for storage and loss moduli.

4.3. RESULTS AND DISCUSSION

4.3.1. Effect of temperature and shear on viscosity

The viscosity of LAB and PP gum solutions (1%, w/w) as a function of shear rate at temperatures of 0.1-75°C is shown in Figures 4.1 and 4.2, respectively; Power Law coefficients for viscosity curves in the range 0.1-75°C are presented in Tables 4.2 and 4.3, respectively.

As expected, viscosity increased with a drop in temperature below 20°C. At higher temperatures of 25-75°C (Figs. 4.2B and 4.3B), viscosity decreased with temperature for both PP and LAB gum solutions without any unusual behavior as described by Dawkins and Nnanna (1995) or Gomez et al (1997). A possible reason for the deviation in the behavior described by Dawkins and Nnanna (1995) may be the initial incomplete hydration

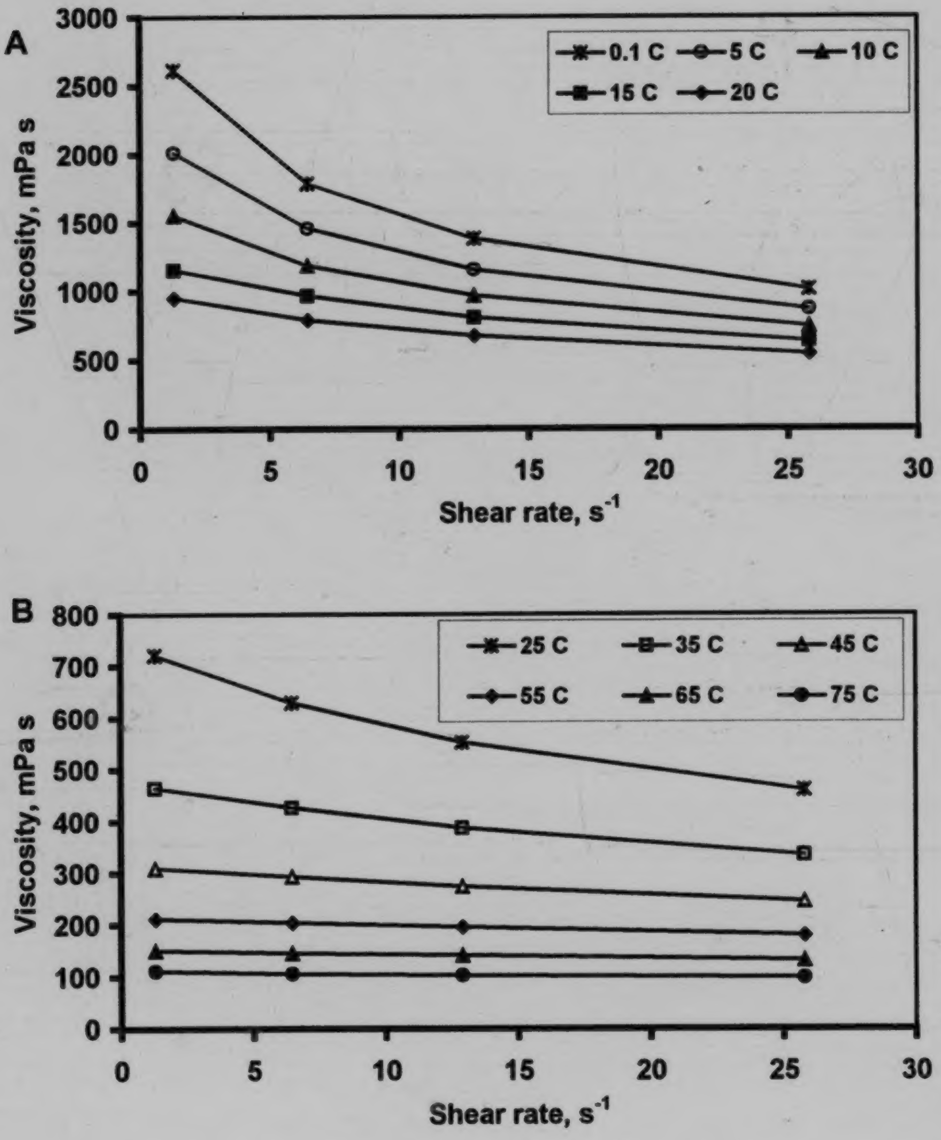

Figure 4.1. Viscosity of 1% (w/w) LAB gum solution at 0.1-20°C (A), 25-75°C (B) determined at 1-20 rpm.

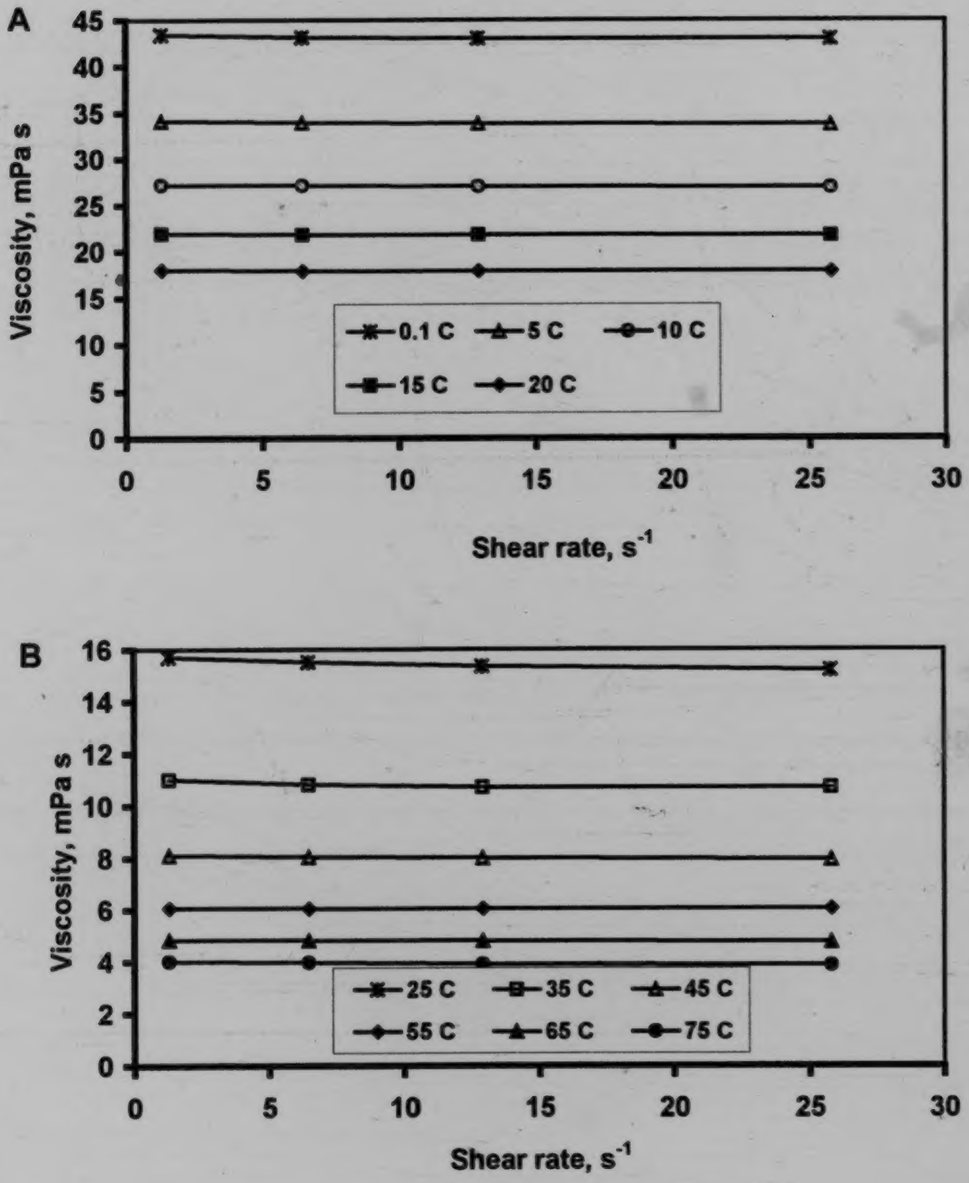

Figure 4.2. Viscosity of 1% (w/w) PP gum solution at 0.1-20°C (A), 25-75°C (B) determined at 1-20 rpm.

TABLE 4.2
Power Law Constants for 1% (w/w) LAB Gum Viscosity Curves in the Range 1-20 rpm (1.29-26.9 s^{-1}) at 0.1-75°C.

Temperature, °C	Consistency Coefficient, c	Flow Behavior Index, n	R^2
0.1	2.9503	0.6899	0.9945
5	2.2486	0.7264	0.9952
10	1.7192	0.7607	0.9957
15	1.2704	0.8075	0.9958
20	1.0324	0.8197	0.9968
25	0.7745	0.8571	0.9977
35	0.491	0.8967	0.9984
45	0.3132	0.9364	0.9988
55	0.2176	0.9494	0.9994
65	0.1544	0.9557	0.9998
75	0.1103	0.9659	0.9999

TABLE 4.3
Power Law Constants for 1% (w/w) PP Gum Viscosity Curves in the Range 1-20 rpm (1.29-26.9 s^{-1}) at 0.1-75°C.

Temperature, °C	Consistency Coefficient, c	Flow Behavior Index, n	R^2
0.1	0.0434	0.9961	1
5	0.0341	0.9961	1
10	0.0272	0.9964	1
15	0.0219	0.9972	1
20	0.018	0.9966	1
25	0.0145	0.9974	1
35	0.0105	0.9955	1
45	0.0078	0.9952	1
55	0.0058	0.9977	1
65	0.0048	0.9945	1
75	0.0039	0.9865	1

of HV oat gum and further swelling of β-glucan micelles upon heating to 37°C. That trend continued up to 61°C, offsetting the drop in viscosity due to temperature rise, while further heating to 100°C resulted in a normal viscosity decrease (Dawkins and Nnanna 1995). Higher viscosity of BBG at low shear rates and 70°C, as described by Gomez et al (1997), is more of an anomaly and could be due to experimental error. Whether their instrument was equipped with the Automatic Gap Control (AGC) feature was not indicated. AGC automatically adjusts the position of measuring elements that may have changed due to thermal expansion. As well, Gomez et al (1997) did not indicate taking any precautions to prevent sample evaporation on the cone edge, which is the most probable reason for the deviation. At elevated temperatures, evaporation of β-glucan solution creates a skin-like layer if the solution is not mixed vigorously and kept in a high humidity environment. This skin-like layer probably contributed to higher viscosity readings at low shear rates, while at higher shear it was disrupted and viscosity readings were closer to expected. Therefore, the sample must be covered with oil for tests at elevated temperatures as well as for tests with a longer time component, even at room temperature. The observed reversible drop in viscosity with temperature may be useful in industrial processing of β-glucan since it enables easier pumping and stirring leading to reduced energy consumption. Despite the change in temperature, the flow behavior index was >0.99 for PP gum solutions in the temperature range 0.1-65°C (Table 4.3), while for LAB gum solutions flow behavior index increased from 0.69 at 0.1°C to 0.966 at 75°C (Table 4.2).

The concentration effect on viscosity of 0.25, 0.5 and 1% LAB gum solutions, and 1, 2.5 and 5% (w/w) PP and Condor gum solutions at 20°C is depicted in Figure 4.3. Viscosity of HV LAB solution increased from 7.9 mPa s at 0.25% to 976 mPa s at 1%

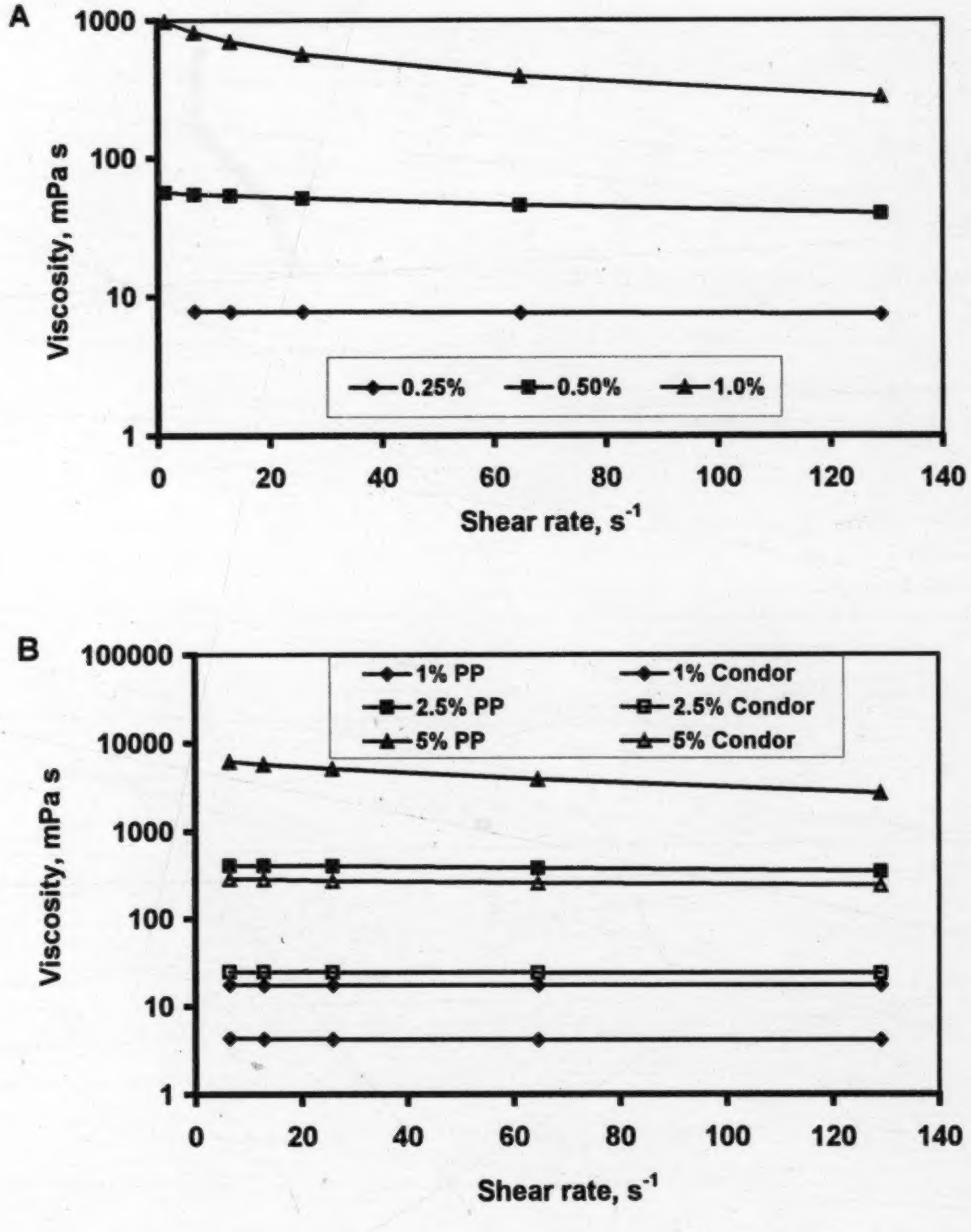

Figure 4.3. Concentration dependence of viscosity vs. shear rate for (A) LAB gum, and (B) Condor (open symbols) and PP gum solutions (solid symbols).

at 1 rpm (1.29 s^{-1}). When the concentration of LV Condor gum solution was increased from 1% to 5%, viscosity increased from 4.3 mPa s to 284 mPa s at 5 rpm (6.45 s^{-1}), while the viscosity of the more viscous PP gum increased from 18 mPa s to 6080 mPa s in the same concentration range and at the same shear rate. The viscosity of a fresh 5% Condor solution at 5 rpm (6.45 s^{-1}) increased from 284 mPa s to 330 mPa s in 14 min (17%) after three consecutive measurements. That was an early indication of network formation.

Laboratory gum exhibited Newtonian behavior at 0.25% concentration with flow behavior index >0.99, while at higher concentrations (0.5-1.0%) its behavior was pseudoplastic. Similarly, PP and Condor gum solution behaved as Newtonian fluids at the lowest tested concentration (1%), whereas at 2.5 and 5.0% concentration both gum solutions were pseudoplastic. At 2.5% concentration, Condor solution was almost Newtonian with the flow behavior index n=0.984.

Arrhenius plots of the viscosity of a 1% LAB gum solution are shown in Figure 4.4, while activation energies at different shear rates and at temperatures between 0.1-75°C are presented in Table 4.4. The decrease in E_a with shear rate for HV LAB gum was in agreement with Autio et al (1987). Pilot Plant gum solution had a constant E_a independent of shear rate, since it exhibits Newtonian fluid behavior (Fig. 4.2). Good agreement (R^2) with linearity was found. Higher E_a means higher viscosity dependence on temperature changes. The activation energy of β-glucan is concentration and shear dependent (Autio et al 1987) and both variables should be reported.

4.3.2. Thixotropy

Thixotropy was not detected for either BBG gum despite the very high shear being applied, which reached approximately 3,800 s^{-1} for LAB gum and approximately 1,300 s^{-1}

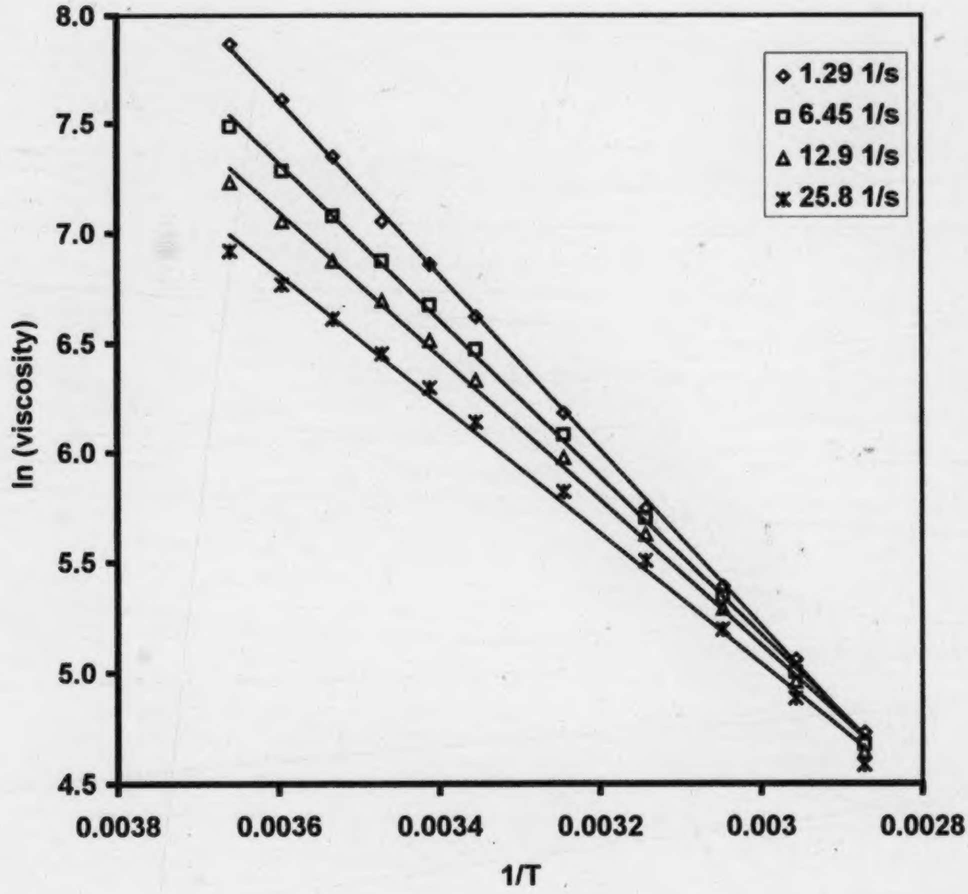

Figure 4.4. Arrhenius plot for viscosity of 1% (w/w) LAB gum solution over the temperature range of 0.1-75°C and at four shear rates (1.29-25.8 s^{-1}).

TABLE 4.4

Activation Energy Equations and Coefficients Calculated from Arrhenius Plot for 1% PP and LAB Gums at 0.1-75°C .

Shear Rate, s^{-1}	Equation	Ea, kJ/mol	A, mPa s	R^2
LAB				
1.29	y=3997.1x - 6.7823	33.23	0.0001	0.9995
6.45	y=3585.2x - 5.5860	29.81	0.004	0.9992
12.9	y=3296.2x - 4.7681	27.40	0.008	0.9979
25.8	y=2962.2x - 3.8498	24.63	0.021	0.9963
PP				
1.29	y=3099.5x - 7.6523	25.77	0.0005	0.9956
6.45	y=3103.8x - 7.6720	25.80	0.0005	0.9959
12.9	y=3116.8x - 7.7197	25.91	0.0004	0.9963
25.8	y=3123.1x -7.7452	25.96	0.0004	0.9964

for PP gum (Figs. 4.5 and 4.6, respectively). This is in agreement with Autio et al (1987) and Wikstrom et al (1994). The time required for the recovery of LAB samples from 3,000 rpm to the starting viscosity at 1 rpm exceeded 4 min without any resting, which confirms the necessity for step wise measurements, especially for HV samples. Pilot Plant gum recovered its original viscosity almost instantly (in <12 s), which is necessary for the next measurement point at 5 rpm. Neither PP gum at 1% nor LAB gum at 0.25% concentration could be tested for thixotropy at 3000 rpm (Fig. 4.7). Laboratory gum (0.25%) was apparently more viscous already at 1000 rpm. It seems that this deviation is viscosity dependent and LV samples have a much lower maximal shear rate limit. When shear rate was adjusted to the initial value of 5 rpm, viscosity curves for both samples shot upward, probably because of low resistance in the sample and the occurrence of turbulent flow when the shear rate was increased from 1000 to 3000 rpm.

The conclusion of Lineman and Kruger (1997) about thixotropy of beer β-glucan should be accepted with great caution and preferably called gel destruction. Their findings should be confirmed using step-wise measurements.

4.3.3. Network formation

Storage modulus G' and loss modulus G'' measurements over time confirmed the β-glucan network formation for LV gums within a 24 h timeframe but at an even lower concentration than that determined visually by Burkus (1996). The time corresponding to the intersection of G' and G'' curves was taken as the time necessary for network formation. The quick gelling LV Condor gum at 5% concentration needed approximately 1.5 h to form a network (Fig. 4.8), but when the concentration was decreased to 2.5%,

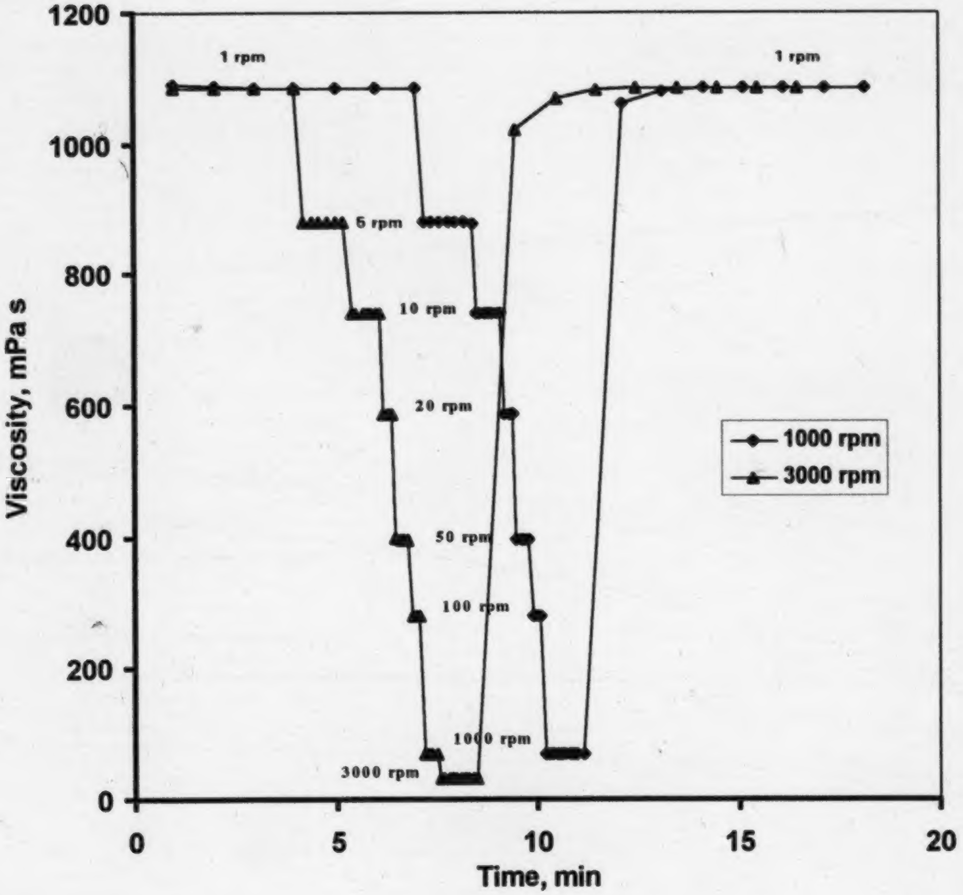

Figure 4.5. Thixotropy of 1% (w/w) LAB gum solution after shearing at 1000 and 3000 rpm at 20°C.

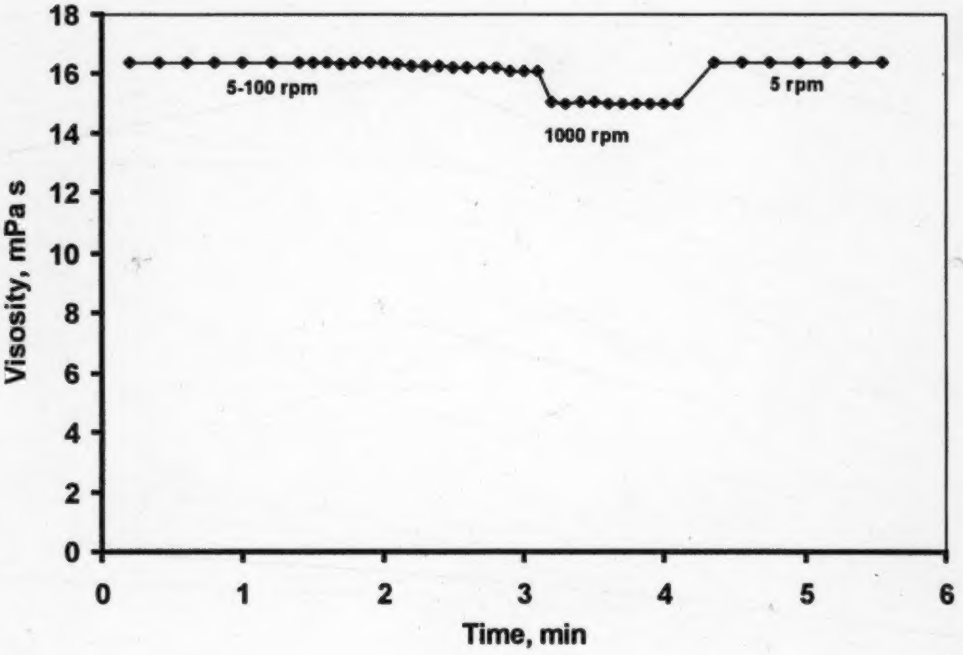

Figure 4.6. Thixotropy of 1% (w/w) PP gum solution after shearing at 1000 rpm and 20°C.

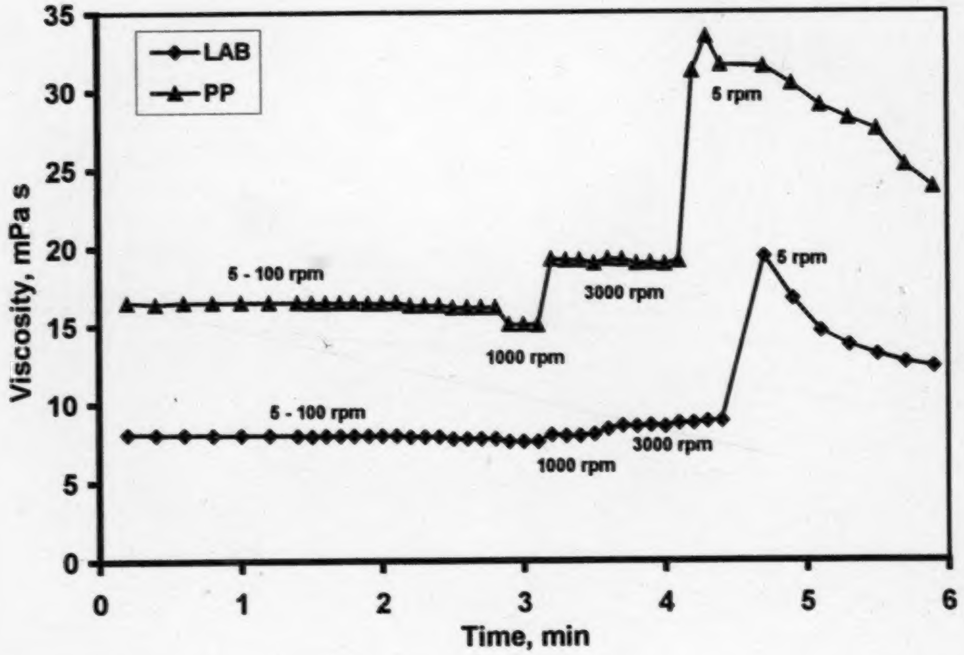

Figure 4.7. Thixotropy of 1% PP gum and 0.25% LAB gum solutions after shearing at 1000 and 3000 rpm and 20°C .

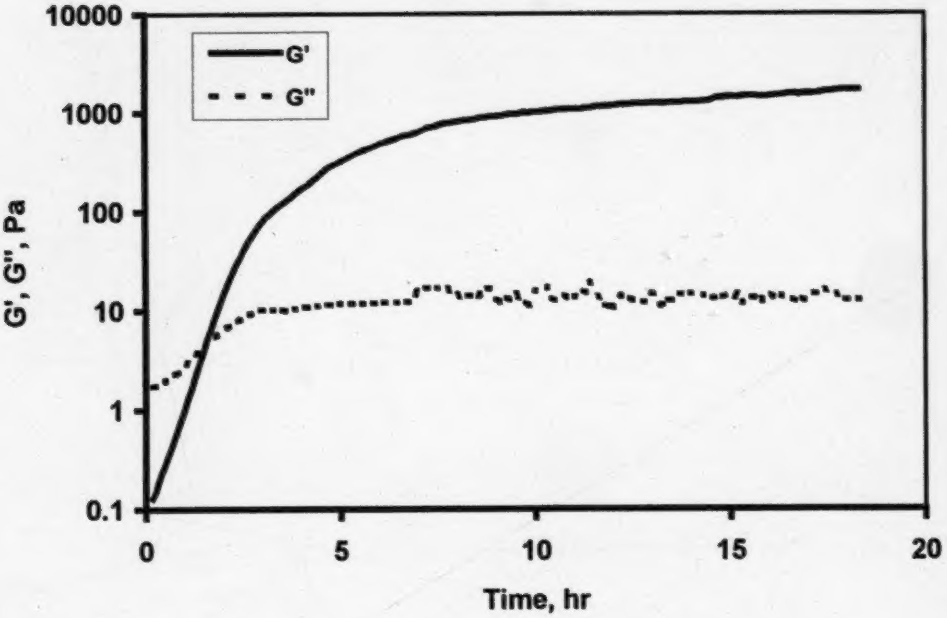

Figure 4.8. Storage modulus (G') and loss modulus (G'') development vs. time for a 5% (w/w) solution of Condor gum determined at 5% strain and 1 Hz frequency. G' exceeds G'' at approximately 1.5 h.

time to gel increased to approximately 13.5 h (Fig. 4.9). In a repeated test, gelation time was approximately 18 h. The first sample was used in some other tests prior to overnight testing of G' and G'', while the second sample underwent testing immediately after hydration and cooling. It seems that the history of a sample plays a significant role in network formation and must be taken into account. At the end of 18 h testing of the 2.5% solution, G' was still increasing while G'' reached a plateau, which implies continued gel strengthening. At the 1% level, Condor gum exhibited a strong gelling tendency, with both G' and G'' significantly increasing overnight, whereas more viscous PP gum had flat values during the test.

Pilot Plant gum at 5% concentration behaved similarly to Condor gum, creating a network in approximately 6.2 h (Fig. 4.10). This is slower than the gelation time for 5% Condor gum (approximately 1.5 hr), which confirms the observations of Burkus and Temelli (1999). Pilot Plant gum is more viscous than Condor gum (18.6 vs. 4.9 mPa s for a 1% solution, Fig. 4.3) and higher viscosity inhibits gelation. That is even more apparent for 2.5% PP gum, which did not form a network within 24 h, whereas the less viscous Condor gum exhibited network formation at 13.5 h at the same concentration (Fig. 4.9). For the 2.5% PP gum, G' increased from 0.06 to 0.11 Pa, while G'' stayed almost constant at approximately 2.6 Pa over 24 h. Less dense packing of the hydrocolloid in the 2.5% solution combined with higher viscosity extends gelling time and probably days, if not weeks, would be necessary for G' to surpass G''. The effect of further dilution was noticeable for 1% PP gum. Although some association was evident from the increase in G' at 2.5% concentration, at 1% there was no significant change in G' throughout the overnight test.

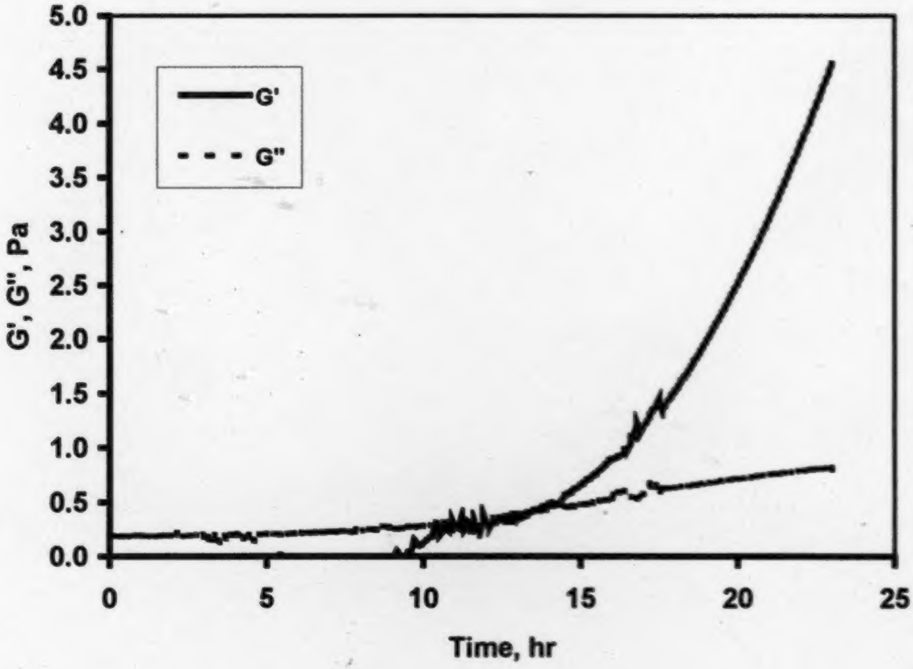

Figure 4.9. Storage modulus (G') and loss modulus (G'') development vs. time for a 2.5% (w/w) solution of Condor gum determined at 5% strain and 1 Hz frequency. G' exceeds G'' at approximately 13.5 h.

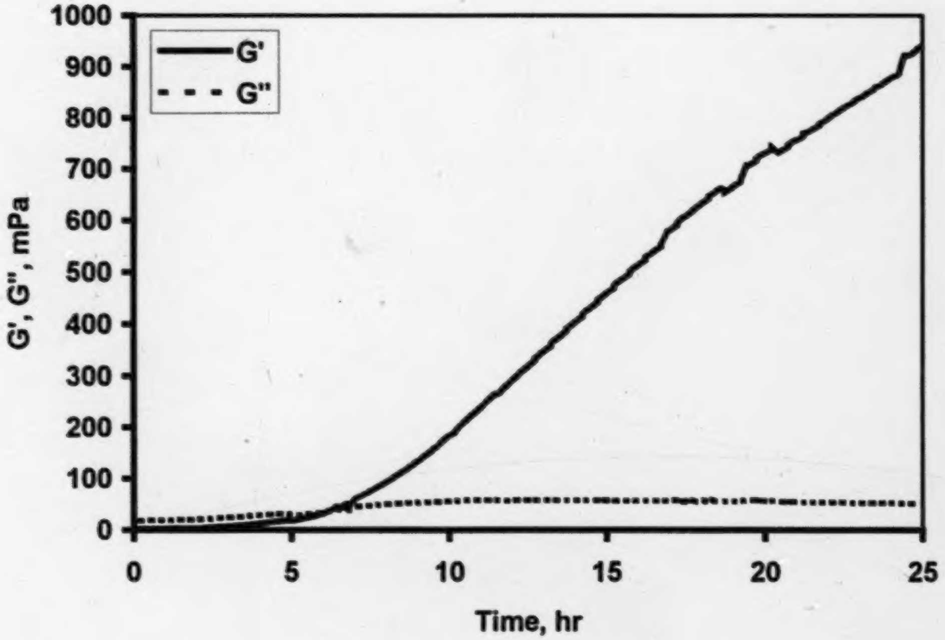

Figure 4.10. Storage modulus (G') and loss modulus (G'') development vs. time for 5% (w/w) PP gum solution determined at 3% strain and 0.5 Hz frequency. G' exceeds G'' at approximately 6.2 h.

High viscosity LAB gum at 1% concentration did not form a network giving no changes in G' and G" within 24 h. Overnight testing at 3% strain and 0.5 Hz resulted in straight lines for $G' \approx 2 \times 10^{-7}$ Pa and $G'' \approx 1.92$ Pa. Laboratory gum was not tested at higher concentrations because PP gum at 2.5% already had extended gelling time in excess of 24 h and LAB gum, which is HV type, will probably require months for gel formation. Increasing concentration would also increase viscosity and, as discussed above, HV is detrimental for gel setting. A sterilized sample of 1% LAB gum left sitting in a refrigerator for more than 6 months had no apparent change in consistency; however, after 1.5 years a weak amorphic gel-like structure was apparent.

4.3.4. Yield point

Yield point was tested for all gum solutions which showed at least some network formation (Figs. 4.11-4.13, Table 4.5). In fresh solutions of BBG gum there was no yield point detectable with this kind of test, even in the case of a quick gelling gum like Condor at 5% concentration (Fig. 4.11). G' and G'' for fresh 2.5% Condor and 5% PP solutions were not shown to preserve the clarity of figures, but exhibited a pattern similar to that of 5% Condor solution with constant G' and G''. Upon standing, the network is formed and G' exceeds G''. After some time G'' reaches a plateau, while G' continues to grow (Figs. 4.8-4.10). During an amplitude sweep, G' continues to grow until stress exceeds the strength of network structure, when G'' again exceeds G' (Figs. 4.11-4.13).

Less concentrated 2.5% Condor gum had G' at the yield point 2 orders of magnitude lower than that of 5% Condor gel (Table 4.5). The strain at the breaking point of the 2.5% gel was >60%, while the 5% gel cracked at approximately 26% strain (Figs. 4.11 and 4.12). Pilot Plant gel (5%) broke at approximately 42% strain (Fig. 4.13), exhibiting

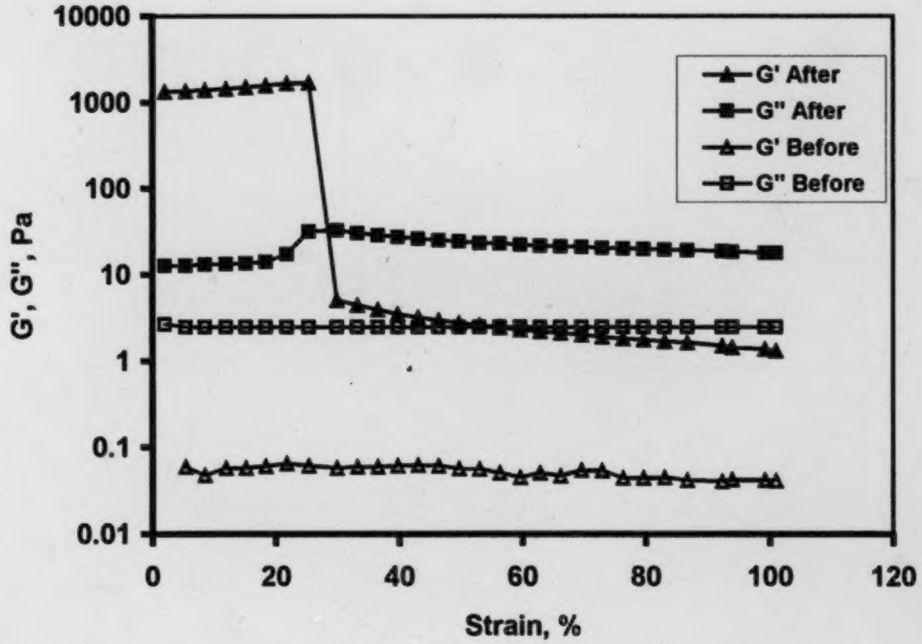

Figure 4.11. Amplitude sweep at 2-100% strain and 1 Hz frequency for 5% (w/w) Condor gum before and after overnight test.

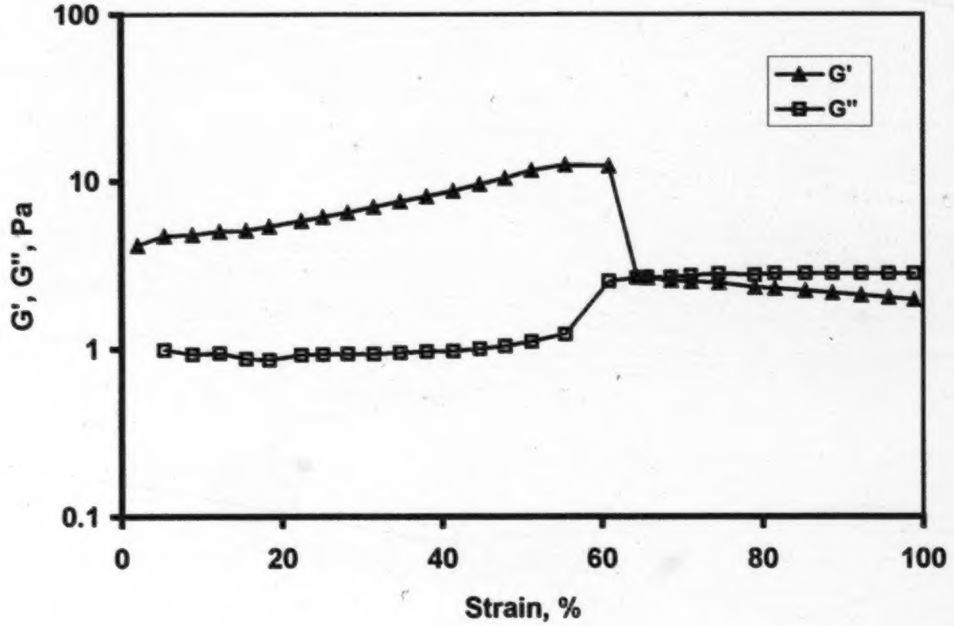

Figure 4.12. Amplitude sweep at 2-100% strain and 1 Hz frequency for 2.5% (w/w) Condor gum after overnight test.

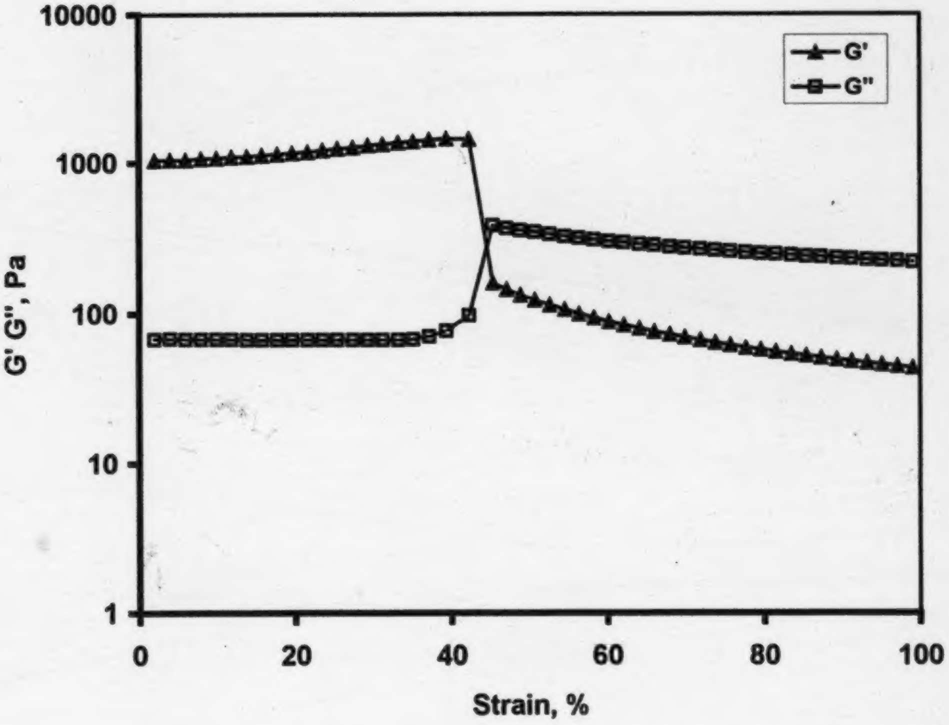

Figure 4.13. Amplitude sweep at 2-100% strain and 1 Hz frequency for 5% (w/w) PP gum after overnight test.

TABLE 4.5
Yield Point Data for PP and Condor Gels After Overnight Setting. Amplitude Sweep was 2-100% Strain at 1 Hz Frequency.

Gum and Concentration	Strain, %	Stress, Pa	G', Pa	G'', Pa
PP, 5%	42.3	607	1430	97
Condor, 5%	25.3	420	1660	17.3
Condor, 2.5%	60.9	7.62	12.3	1.22

higher elasticity than the 5% Condor gel, which could be a consequence of more flexible, longer polymeric chains. Pilot Plant gum at 2.5% did not have a measurable yield point after overnight aging despite some association registered through increased G'.

4.4. CONCLUSIONS

High viscosity LAB gum at 1% concentration is highly pseudoplastic but with a relatively high flow behavior index of >0.8 above 15°C. For HV LAB gum, activation energy is inversely proportional to shear rate showing lower temperature dependence of viscosity at increased shear rates. Conversely, PP gum was Newtonian with a flow behavior index >0.99 for temperatures of 0.1-75°C. The activation energy for PP gum was independent of shear rate. Fresh solutions of β-glucan were not thixotropic at concentrations ≤1%, regardless of viscosity.

Network formation in solutions of LV β-glucan is highly time and concentration dependent. The less viscous Condor gum at 5% concentration had already set a gel after 1.5 h of testing when G' exceeded G''. Decreasing the concentration by half extended the gelling time by at least 9-fold. More viscous PP gum at the same 5% concentration needed at least 6.2 h to gel, while at 2.5% concentration, PP gum did not create a continuous network overnight. Using the same ratio (9-fold) for the effect of concentration on time necessary for gel setting as for Condor gum, about 2.5 days would be necessary for the gelling of 2.5% PP solution. The higher viscosity of PP gum should be an additional factor slowing down network formation. Laboratory gum at ≤1% concentration showed no gelling tendency in 24 h and was expected to stay as such over a period of a few months.

Yield point could not be detected in fresh β-glucan solutions with the testing mode applied, regardless of viscosity or concentration, but solutions having a continuous network in which G' exceeded G'', exhibited a yield point.

4.5. REFERENCES

Autio, K., Myllymaki, O., and Malkki, Y., 1987. Flow properties of solutions of oat β-glucans. J. Food Sci. 52:1364-1366.

Autio, K., Malkki, Y., and Virtanen, T. 1992. Effects of processing on the microstructure of oat (*Avena sativa*) bran concentrate and the physicochemical properties of isolated β-glucans. Food Structure, 11:47-54.

Beer, M. U., Wood, P. J., and Weisz, J. 1997. Molecular weight distribution and (1→3)(1→4)-β-D-glucan content of consecutive extracts of various oat and barley cultivars. Cereal. Chem. 74:476-480.

Bhatty, R. S. 1995. Laboratory and pilot plant extraction and purification of β-glucans from hull-less barley and oat bran. J. Cereal Sci. 22:163-170.

Burkus, Z. 1996. Barley β-glucan: Extraction, Functional Properties and Interaction with Food Components. M. Sc. Thesis. University of Alberta, Edmonton, AB.

Burkus, Z., and Temelli, F. 1998. Effect of extraction conditions on yield, composition and viscosity stability of barley β-glucan gum. Cereal Chem. 75:805-809.

Burkus, Z., and Temelli, F. 1999. Gelation of barley β-glucan concentrate. J. Food Sci. 64:198-201.

Cui, W., Wood, P. J., Weisz, J., and Beer, M. U. 1999. Nonstarch polysaccharides from preprocessed wheat bran: Carbohydrate and novel rheological properties. Cereal Chem. 76:129-133.

Dawkins, N. L., and Nnanna, I. A. 1995. Studies on oat gum [(1→3, 1→4)-β-D-glucan]: composition, molecular weight estimation and rheological properties. Food Hydrocol. 9:1-7.

Doublier, J. L., and Wood, P. J. 1995. Rheological properties of aqueous solutions of (1→3) (1→4)-β-D-glucan from oats (*Avena sativa* L.). Cereal Chem. 72:335-340.

Gomez, C., Navarro, A., Garnier, C., Horta, A., and Carbonell, J. V. 1997. Physical and structural properties of barley (1→3),(1→4)-β-D-glucan III. Formation of aggregates analyzed through its viscoelastic and flow behavior. Carbohydr. Polym. 34:141-148.

Linemann, A., and Kruger, E. 1997. Relationships between structure and characteristics of β-glucan in beer production. Part 1: Properties in dissolving β-glucan out of beer. Brauwelt Int. 1997(IV):344-351.

Morgan, K. R., and Ofman, D. J. 1998. Glucagel, a gelling β-glucan from barley. Cereal Chem. 75:879-881.

Speers, A. 1999. Personal communication. Department of Food Science and Technology Daltech, Dalhousie University, Halifax, NS.

Szczesniak, A. S., and Farkas, E. 1962. Objective characterization of the mouthfeel of gum solutions. J. Food Sci. 27:381-385.

Wikstrom, K., Lindahl, L., Andersson, R., and Westerlund, E. 1994. Rheological studies of water-soluble (1→3), (1→4)- β-D-glucans from milling fractions of oats. J. Food Sci. 59:1077-1080.

Wood, P. J., Weisz, J., Fedec, P., and Burrows, V. D. 1989. Large scale preparation and properties of oat fractions enriched in (1→3)(1→4)-β-D-glucan. Cereal. Chem. 66:97-103.

Wood, P. J., Weisz, J., and Mahn, W. 1991. Molecular characterization of cereal β-glucans II, size exclusion chromatography for comparison of molecular weight. Cereal Chem. 68:530-536.

5. INFLUENCE OF PREPARATION, SHEAR AND STORAGE TREATMENTS ON BARLEY β-GLUCAN SOLUTIONS[1]

5.1. INTRODUCTION

Surplus water used for hydration of hydrocolloids may influence the final viscosity of a prepared solution. Morin and Temelli (unpublished data) dissolved carboxymethylcellulose at 4% concentration. The solution which was, prepared with approximately 50% surplus water and surplus water evaporated prior to cooling, was apparently thicker and gel-like, while the solution prepared with the exact amount of water was easily pourable. It seems that surplus water may allow more complete hydration of molecules in solutions with a high concentration of hydrocolloids (>1%). It may be expected that at a concentration of 1%, low viscosity (LV) barley β-glucan gum (BBG) would not be much different in viscosity due to water surplus, but high viscosity (HV) gum may exhibit minor changes. At higher concentrations (i.e. ≥2.5%), LV gum may also be expected to have higher viscosity with surplus water. The information about the amount of water necessary for more complete hydration may be valuable for industrial preparation and utilization of β-glucan gums.

Cooling rate played a role in the viscosity of beer worts. Lineman and Kruger (1998) found that wort cooled from 78 to 20°C in 3 min had a higher intrinsic viscosity than a sample cooled over 15 min. Their explanation was that quickly cooled β-glucan could form micelles in random fashion, while slowly cooled chains had enough time to align themselves and even precipitate from solution. Similar behavior was observed with amylose solutions (Glicksman 1969). It is not known if cooling speed affects the viscosity of β-glucan that is more viscous than beer β-glucan, or at the higher concentrations normally encountered in food applications of hydrocolloids.

---

[1] A version of this chapter is to be submitted to Journal of Food Science for consideration for publication.

Shear was responsible for a decrease in viscosity during pilot plant extraction of oat β-glucan (Wood et al 1989). The gum obtained in the pilot plant (PP) had lower viscosity than that produced in the laboratory (LAB) under similar conditions but devoid of the high shear of pilot plant centrifugal separators. Laboratory gum was subsequently sheared in an Oster blender and Polytron homogenizer, which resulted in >60% lower viscosity after both treatments (Wood et al 1989). Despite that finding, Dawkins and Nnanna (1995) used a Polytron to homogenize oat gum in binary systems with other food gums. Whether the use of Polytron influenced viscosity was not indicated since the focus was on the prevention of particle settlement upon solubilization of gums in hot water. Harrington and Zimm (1965) found that DNA was degraded more rapidly in 50% glycerol solution with few times higher viscosity than that of a water solution, at a 20% lower blade speed. Ullrich et al (1986) extracted BBG using sonication in an ultrasonic cleaner for 1 h. The type of ultrasonicator and the details of the procedure (generator type and energy) were not reported. Ultrasonication is known to degrade polymers of bacterial origin (Szu et al 1986) and may also be detrimental to β-glucan.

Freeze-thaw stability of β-glucan has not been reported in the literature, although Morgan and Ofman (1998) used freezing at -10°C as a means to precipitate β-glucan from solution. Freeze-thaw stability may be important for β-glucan stability upon unintentional freezing as well as applications in frozen food products. Therefore, it merits further investigation.

Long term storage stability of β-glucan solutions has not been reported in the literature. However, Burkus and Temelli (unpublished data) noticed that 1% LV BBG gum solutions would set into a soft gel after prolonged storage at room temperature.

Therefore, the objectives of this study were:

- to test the effect of the water surplus used during preparation on final solution viscosity,
- to examine if cooling rate affects the final viscosity of β-glucan solutions,
- to investigate the effect of Polytron and ultrasonic shear on the viscosity of BBG,
- to analyze the influence of freeze-thaw cycles on β-glucan viscosity, and
- to test LAB and PP BBG solutions stability over a 4 week period.

5.2. MATERIALS AND METHODS

5.2.1. Materials

Pilot Plant and LAB gums were obtained as described in Chapter 2 and solutions were prepared at few desired concentrations (w/w) based on Burkus and Temelli (1998). Solutions were brought to boiling, held 1 h at approximately 80°C with stirring and cooled to room temperature. The weight was adjusted by adding distilled water to make up for evaporation losses. Deviations from this procedure are described under methods, since some preparation techniques were studied as processing variables. Distilled water from the local supply was used for the preparation of BBG solutions.

5.2.2. Treatment methods

Surplus water was added in an amount 50% greater than that necessary to obtain a desired concentration, while the blank contained the exact amount of water ±5%, i.e. blank sample used 10 g of water to prepare a 1% solution vs. 15 g as an experimental treatment. Beakers were tightly covered with aluminum foil and β-glucan was dissolved according to Burkus and Temelli (1998). Halfway through the 1 h holding period at ≈80°C and when β-glucan appeared to be completely dissolved, beakers with surplus water were uncovered and the surplus water was evaporated with vigorous stirring on a magnetic stirrer to enhance evaporation and prevent formation of a skin-like layer on the top of the solution. Then, viscosity at a fixed speed of 10 rpm (12.9 s^{-1}) was determined as described below. Each gum, at 1% (w/w) concentration, was tested in duplicate.

The effect of cooling rate was tested by splitting hot BBG solution into two parts and recording their weights. One sample was quickly cooled in an ice bath, while the second sample was placed in a hot waterbath at 75°C without stirring. Temperature was decreased 1°C every 10 min to allow enough time for possible linear association of β-glucan molecules. When the temperature reached 45°C, the samples were taken out of the waterbath and left on the bench to reach room temperature. The weight of both samples was then adjusted to the initial level and viscosity was measured. Both LAB and PP gum solutions were tested at 1% (w/w) concentration, in duplicate.

Shearing was performed using a Polytron homogenizer (model PT-2000, Kinematica AG, Littau, Switzerland) equipped with a 20 mm toothed generator at speed 1 for 1 min. Ultrasonication was carried out for 60 s with a Sonic 300 Dismembrator (Artek Systems Corp., Farmingdale, NY) equipped with an intermediate tip at 60% power - the maximum allowed for that kind of tip. Viscosity was recorded, samples were reheated to >60°C to melt any aggregates that may have formed upon shearing, cooled, their weights adjusted, and viscosity was recorded again. Laboratory gum was prepared at 0.7%, 0.5% and 0.25% (w/w) concentration, while PP gum was prepared at 1% and 0.5% (w/w) concentration. Each gum at each concentration was prepared in duplicate.

Freeze-thaw (F-T) stability was tested in 0.5% and 1% (w/w) PP and 0.25%, 0.5% and 1% (w/w) LAB solutions in duplicate through 4 freeze-thaw cycles with overnight freezing at -15°C. After solubilization at the desired concentration (Burkus and Temelli 1998), 25 g of BBG solution was placed in capped plastic tubes (50 mL) and frozen overnight at -15°C . Initially, defrosting was done by manual shaking under running water, but there was an inconsistency in the viscosity readings of duplicate samples, which implied that there may be phase separation. Therefore, the samples were defrosted in tap water without any shaking and a sample was drawn from the very top of the tubes for viscosity determination. The second sampling was done with a partially cut 1 mL pipette tip (to minimize damage to the structure) from the very bottom of the tubes. A viscosity differences between the layers would indicate β-glucan settling. After measurement, sample was returned to the tubes to the region from which it had been taken. Samples were left on the bench for the next 3-4 h to stabilize and recover from the disturbance and then frozen again. Reheating after the 4th F-T cycle was carried out in the way described for sample preparation in a hot water bath at 80°C with intermittent vortexing. All samples were preserved with the common milk preservative Brotab 10 (6 mg of Bronopol and 0.3 mg of Natamyicin per 20 mg tablet; Systems Plus, New Hamburg, ON) using 1 tablet per 25 g of solution.

Simulated "accidental" freezing was tested as a regular 1st F-T cycle as described above with subsequent viscosity measurements over 4 days of refrigerated storage. Pilot Plant and LAB gum were tested as 1% (w/w) solutions. A control sample, which was not subjected to freezing, was stored in the refrigerator.

The effect of long term refrigerated storage was assessed by monitoring viscosity in 1% (w/w) PP and LAB solutions over 4 weeks of storage at 4°C. Control samples for the F-T test were also used for this test. The first set of samples was used for 4 weeks, which means that the samples were disturbed every week during sampling and viscosity measurement and then returned into the sample tubes. The second set of samples was refrigerated over the desired period without any disturbance. Each sample was pulled from storage just before viscosity measurement and discarded after the measurement. Therefore, this set of samples was disturbed only once and the difference in the results from the first set shows the influence of stress on network formation over time. All samples were prepared in duplicate.

5.2.3. Rheological measurements

Viscosity was determined by consecutive fixed speed tests using a PAAR Physica UDS 200 rheometer (Glenn Allen, VA) equipped with a Peltier heating system. Tests were performed at 20±0.02°C using the DG 27 cup and bob geometry with double gap and a 7 mL sample size. Sample size was not measured by volume due to the difficulty of measuring highly viscous samples. Instead, the clean DG 27 cup was placed on the balance, tared and 7.01 ± 0.005 g of sample was weighed directly into the cup. Shear rate data are reported as rpm, or as s^{-1} after multiplication by the conversion factor 1.29.

Yield point was determined in the amplitude sweep-controlled shear displacement (CSD) mode at 20°C, 1 Hz frequency and strain ranging between 0.1-100% or less, depending on the viscosity of the sample and the necessary stress to be applied on the sample, as calculated by the US200 program in the Analysis window.

5.2.4. Scanning electron microscopy (SEM)

Pilot Plant and LAB gum solutions were prepared at concentration of 0.125, 0.500 and 1.000% (w/w), and frozen in liquid nitrogen. The sample surface was sheared off, allowed to sublime in vacuum, sputter coated with gold and then examined under a scanning electron microscope (Jeol-JSM 6301FXV, Akishima, Tokyo, Japan). Magnification ranged from 1000 to 100,000 times. An additional 3% PP solution was stored at 4°C for a week to allow gelling to occur, while 1% PP gum solution was frozen and thawed. Both samples were then examined using SEM following the procedure above.

5.2.5. Statistical analysis

Each solution for every treatment was prepared in duplicate. Viscosity measurements were carried out in triplicate for each sample. Analysis of variance of the results was performed using the General Linear Model procedure of SAS Statistical Software, Version 6 (SAS Institute 1989). Multiple comparison of the means was performed by the least significant difference (LSD) test at $\alpha=0.05$.

5.3. RESULTS AND DISCUSSION

5.3.1. Solution preparation treatments

Surplus water used in the preparation had no significant influence ($p>0.05$) on the viscosity of 1% solutions of PP or LAB BBG. Therefore, at this concentration level, BBG solutions may be prepared with optimal water content, which should take into account evaporation during preparation. Such a procedure should shorten handling time.

Cooling rate, as tested in this study, did not significantly influence ($p>0.05$) the viscosity of 1% PP and 1% LAB gum solutions. This finding indicates that there exists significant flexibility regarding cooling rate in designing β-glucan extraction or preparation methodology in conjunction with industrial processing.

5.3.2. Shear effect

Shear effect was not significant ($p>0.05$) when β-glucan solutions were subjected to thixotropy tests (Chapter 4). The UDS 200 rheometer had a maximum speed of 3,000 rpm corresponding to approximately 3,870 s^{-1}. (Flow was laminar in nature and β-glucan molecules or micelles could be broken by stretching.) However, when the Polytron homogenizer was applied as a means of shear, which was more like grinding in a narrow space and cutting with sharp blades, viscosity decreased by approximately 50% for a HV 0.7% LAB gum solution (Fig. 5.1), but only approximately 20% for the less viscous 0.5% solution (Fig. 5.2) and approximately 15% for LAB gum at 0.25% concentration (Fig. 5.3). If the influence of shear was reversible, reheating or standing should allow recovery of the original β-glucan micellar structure. However, the viscosity drop was not recovered through reheating. There was also no recovery of LAB gum solution viscosity upon standing for 24 h. Surprisingly, the viscosity of LV PP gum was not affected by Polytron treatment at 1% (Fig. 5.4) or 0.5% concentration (Fig. 5.5).

Ultrasonication resulted in a decrease in the viscosity of both LAB and PP gums at all concentrations (Fig. 5.1-5.5). Pilot Plant gum solutions were more resistant to ultrasonication, losing 10-12% viscosity, while LAB gum solutions lost 40-50% of their original viscosity. Again, reheating and holding overnight did not recover the lost viscosity. Pilot Plant samples after Polytron treatment, which were left on the bench for 48 h, developed an off-white precipitate without any viscosity loss and became more transparent indicating precipitation of protein that was denatured or agglomerated and sedimented out

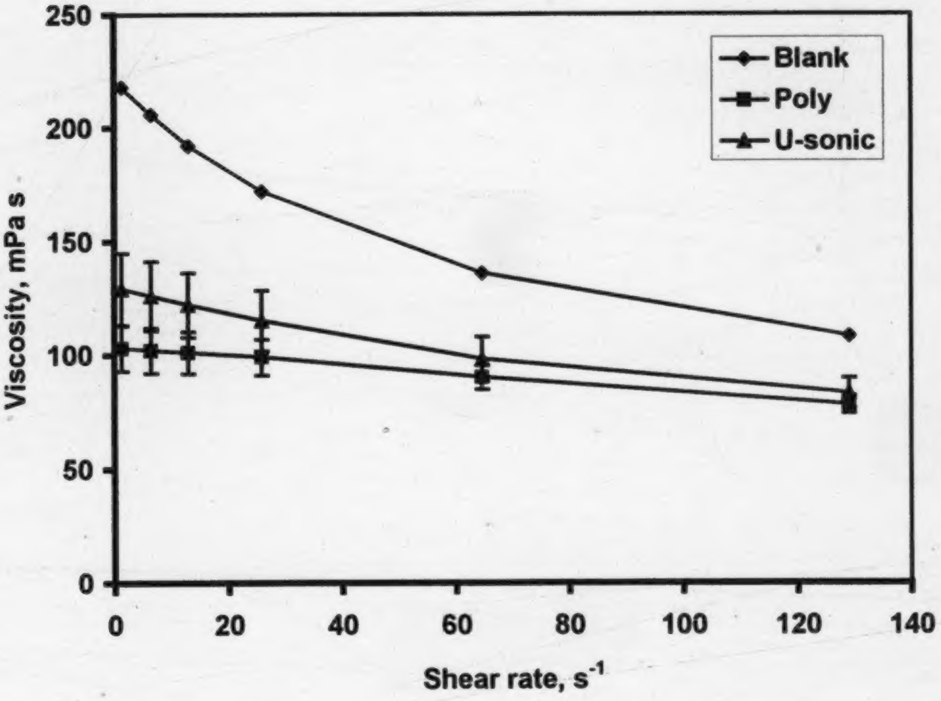

Figure 5.1. The effect of Polytron shear (Poly) and ultrasonication (U-sonic) on the viscosity of 0.7% (w/w) LAB gum after 60 s treatment.

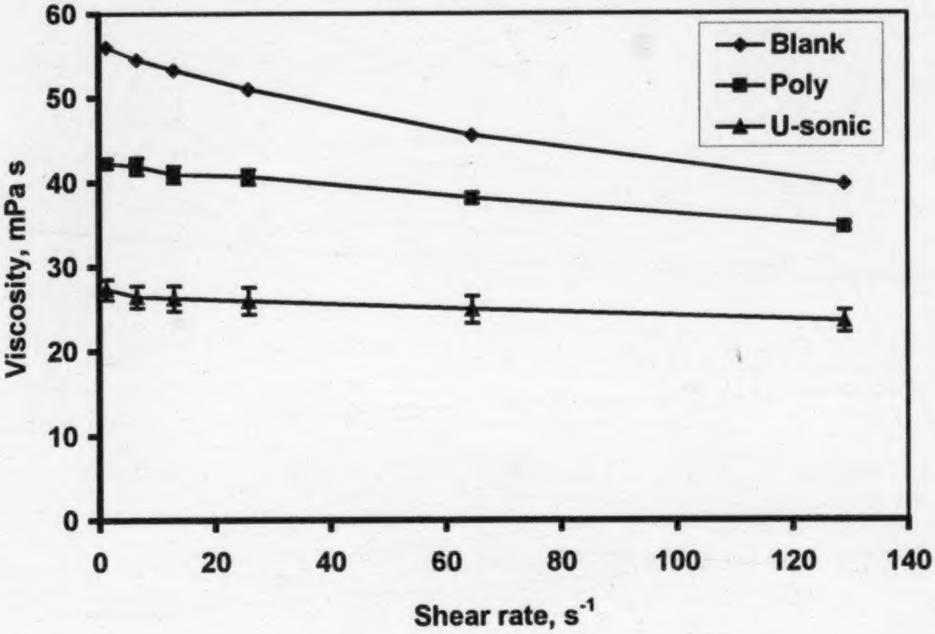

Figure 5.2. The effect of Polytron shear (Poly) and ultrasonication (U-sonic) on the viscosity of 0.5% (w/w) LAB gum after 60 s treatment.

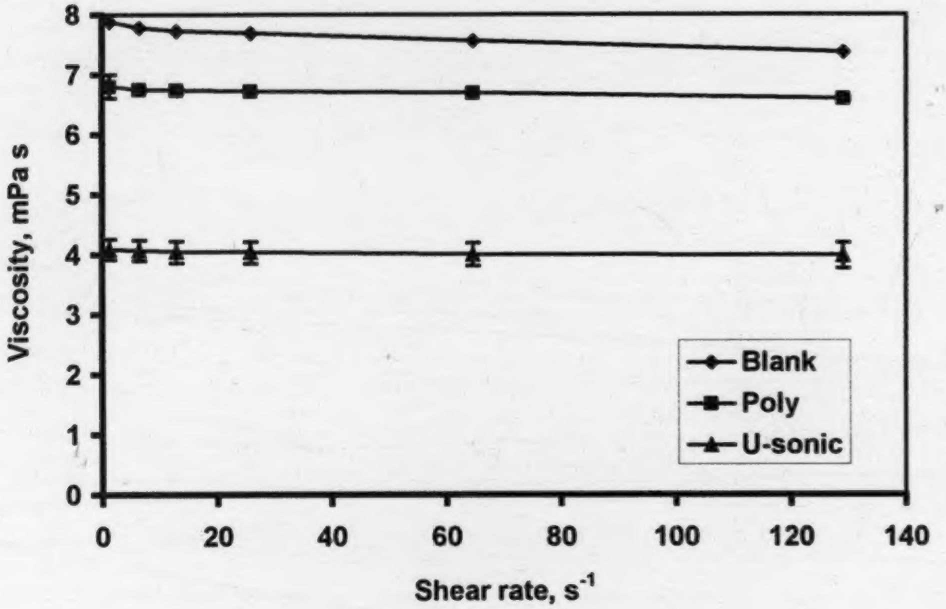

Figure 5.3. The effect of Polytron shear (Poly) and ultrasonication (U-sonic) on the viscosity of 0.25% (w/w) LAB gum after 60 s treatment.

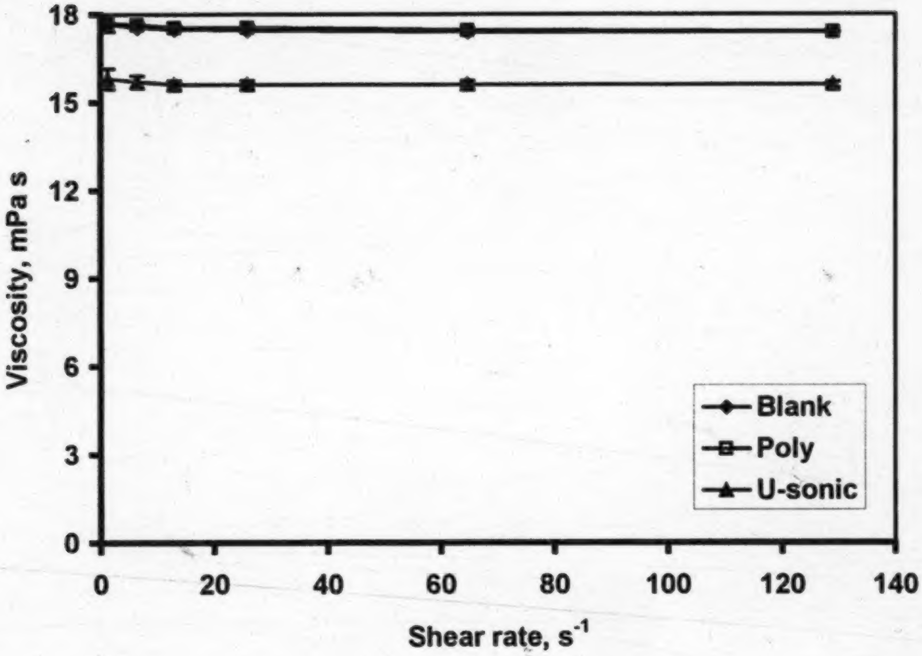

Figure 5.4. The effect of Polytron shear (Poly) and ultrasonication (U-sonic) on the viscosity of 1% (w/w) PP gum after 60 s treatment.

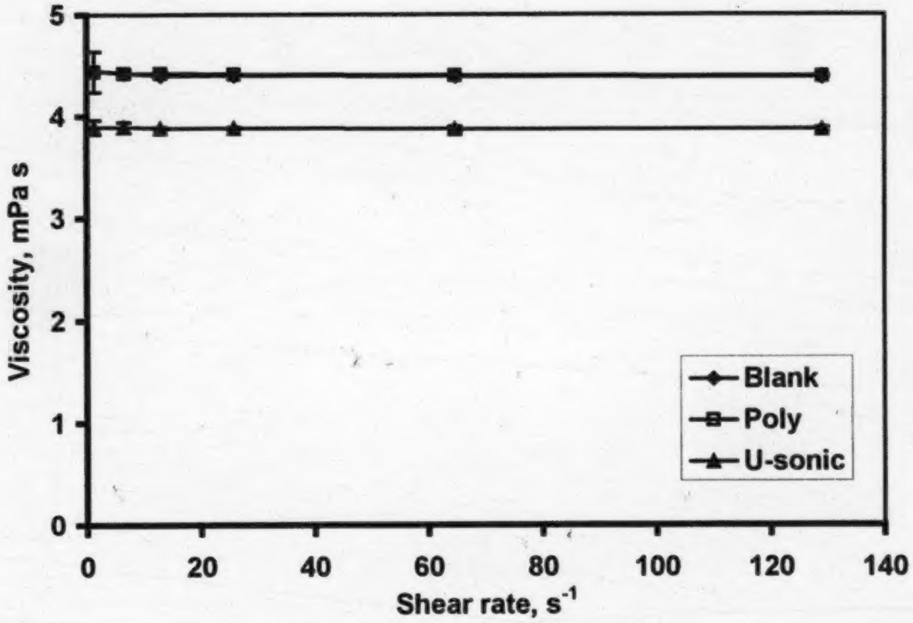

Figure 5.5. The effect of Polytron shear (Poly) and ultrasonication (U-sonic) on the viscosity of 0.5% (w/w) PP gum after 60 s treatment.

of solution. There may be multiple reasons for the different shear resistance of low- and high-viscosity gums. HV and LV gums must have some structural differences, which protect LV PP gum from losing its viscosity after Polytron treatments.

The first possibility may be that HV gum has much longer micellar tails that are sheared off the micelle surface. This would lower viscosity substantially since the micelle's gyration radius would be smaller, and the absence of tails would prevent them from forming agglomerates at low shear rates. The deviation from the Power Law curve for the HV-Polytron treated solution at low shear rates (Fig. 5.1) supports this argument. The second reason for the shear resistance of PP gum could be that its micellar structure is different from that of micelles formed by HV gum. Pilot Plant micelles can be much more compact, smaller in size and contain more β-glucan chains. Protruding tails would be very short and less interacting with other tails in fresh solutions, which gives the solution Newtonian-like flow behavior. These micelles can be envisioned like fuzzy balls that bounce around in solution under high shear. Conversely, HV gum micelles are much larger and fluffier with longer tails. They contain fewer β-glucan chains, which means that they fill up the volume better having a larger gyration radius, resulting in a much higher solution viscosity. Applied shear in the form of cutting and grinding, as is the case with Polytron homogenizer, can affect them to a greater extent and tear micelles. Liberated β-glucan chains can regroup forming smaller more compact micelles, resulting in lower viscosity.

Pilot Plant and LAB gums have low- and high-viscosity, respectively, which is a direct consequence of their low and high molecular weight (Chapter 3). The difference in chain length in addition to the difference in molecular structure, i.e. a higher proportion of

(1→3) bonds in oat β-glucan (Wood et al 1991a) must be responsible for the difference in solution behavior between BBG and OBG. The difference in the structure of BBG and OBG was confirmed by Grimm et al (1995) and Varum et al (1992). BBG forms micelles with 17-70 chains joined into a multimer (Grimm et al 1995). The difference in micellar structure between BBG in beer with shorter chains and OBG with longer chains was also proposed by Grimm et al (1995). The above speculations on micellar structure of β-glucan require further substantiation using image analysis techniques.

The mode of ultrasonic action is obviously different since there was a change in the viscosity of LV PP gum solutions. Ultrasonication was probably able to degrade the micelles further by increasing the number of shorter free flowing chains, which can only form smaller micelles.

The difference in the micellar structure of HV and LV β-glucan, as depicted in the above discussion and implying differences in the protruding micellar tails, can lead to an explanation of the gelling properties for these two types of BBG. At equal concentrations, LV β-glucan contains a larger number of chains in solution than HV β-glucan, which means more endings that can interact with each other over time when certain concentration is achieved. High viscosity oat β-glucan, with twice the molecular weight of LV oat β-glucan, has up to 10 times higher viscosity (Wood et al 1991b) and half as many molecular endings. Still, gel formation is technically not possible with HV β-glucan over a short period of time (e.g. <3 months). The increase in viscosity of such a gum is not enough to retard network formation to such a degree that even a weak gel cannot be formed. Yet, there is some weak interaction among HV β-glucan molecules or micelles, which creates high pseudoplasticity, while LV tails form a network (Chapter 4). A plausible explanation may be that LV β-glucan was formed through alkaline and shear cleavage at the weakest spot, which is the (1→3) bond (Luchsinger and Stone 1976, Gaosong and Vasanthan 2000). That cleavage liberates two new cellulosic (1→4) regions. As well, the peeling reaction (Chapter 2) could degrade more susceptible (1→3) bonds at the reducing ends (White and Kennedy 1988), leaving only cellulosic (1→4) regions at those ends of β-glucan chains. It means that the dominant new β-glucan endings are cellulosic (1→4) regions, which can interact with each other and create either stiffer, compact micelles or connect micelles into a network. Short (1→4) regions, present as endings in native long HV β-glucan molecules, cannot form firm crystalline cellulosic regions, but can exhibit a weak attraction, which creates the molecular or micellar flocks responsible for pseudoplastic behavior. Upon shear, these flocks dissipate relatively easily and reform upon shear cessation - a typical pseudoplastic behavior (Morris 1989).

5.3.3. Freeze-thawing of BBG solutions

Freeze-thaw cycles were detrimental to the viscosity and structure of both LAB and PP gums, with LAB gum showing more resistance. The surface layer of 1% PP gum lost 53% of its apparent viscosity at 10 rpm after the first F-T cycle (Table 5.1), whereas that of the bottom layer increased by about 47%, signaling the beginning of β-glucan settling. The second F-T cycle continued the trend, with visible phase separation into a clear upper layer with viscosity of 5.5 mPa s (approximately 70% lower) and a lower gel-like opaque phase amorphous in appearance. After the third F-T cycle, the upper layer lost its transparency but the change in viscosity was small, while after the fourth F-T cycle, it recovered transparency with a further viscosity drop to 2.7 mPa s. A possible reason for such behavior may be the dual structure of β-glucan as elaborated by Varum et al (1992).

TABLE 5.1
Changes in the Apparent Viscosity of 1% (w/w) PP and LAB Gum Solutions After 4 Freeze-Thaw (F-T) Cycles as Measured at 10 rpm (12.9 s^{-1}) and 20°C.

	Viscosity, mPa s					
Gum	**Fresh**	**1st F-T Bottom**	**1st F-T Top**	**2nd F-T Top**	**3rd F-T Top**	**4th F-T Top**
PP	18.2	26.7	8.47	5.50	4.97	2.65
LAB	693	845	492	386	288	174

Micelles, which are responsible for network formation and impart most of the viscosity, are destabilized by freezing and are forced into network or gel formation due to the concentration effect associated with freezing, while free floating molecular chains are more resistant to that effect and, as a true solution, do not disperse light very much, leading to increased transparency. However, after repeated freezing, some of these chains may be further agglomerated and precipitated, hence the decreased transparency after the third freezing.

LAB gum at 1% concentration was more resistant to freeze thaw cycles with a 29% drop in viscosity of the surface layer after the first F-T cycle (Table 5.1), and with visible settling after the fourth F-T cycle, but without phase separation. Although the viscosity drop was significant after the first F-T cycle, the residual viscosity was still high enough to exert a protective role in a potential food system. Eventual network formation upon freezing without phase separation may even increase the stability of a food product into which β-glucan has been incorporated. The existence of a yield point was detected after the fourth F-T cycle, which showed a yield stress of 0.2 Pa at approximately 3% strain.

Freeze-thaw stability of 0.5% LAB gum solution was similar to that of 1% PP gum (Fig. 5.6), losing about 30% viscosity in the top layer after the first F-T cycle and about 65% after four F-T cycles, while the bottom layer exhibited an opposite trend, gaining about 30% in viscosity after the 4 F-T cycles. Thus, it can be concluded that higher viscosity did have a protective effect against freezing. After four F-T cycles, settling in the bottom layer was barely visible. Reheating of 0.5% and 1% LAB gum solutions recovered viscosity completely, indicating a reversible change in the physical structure of BBG.

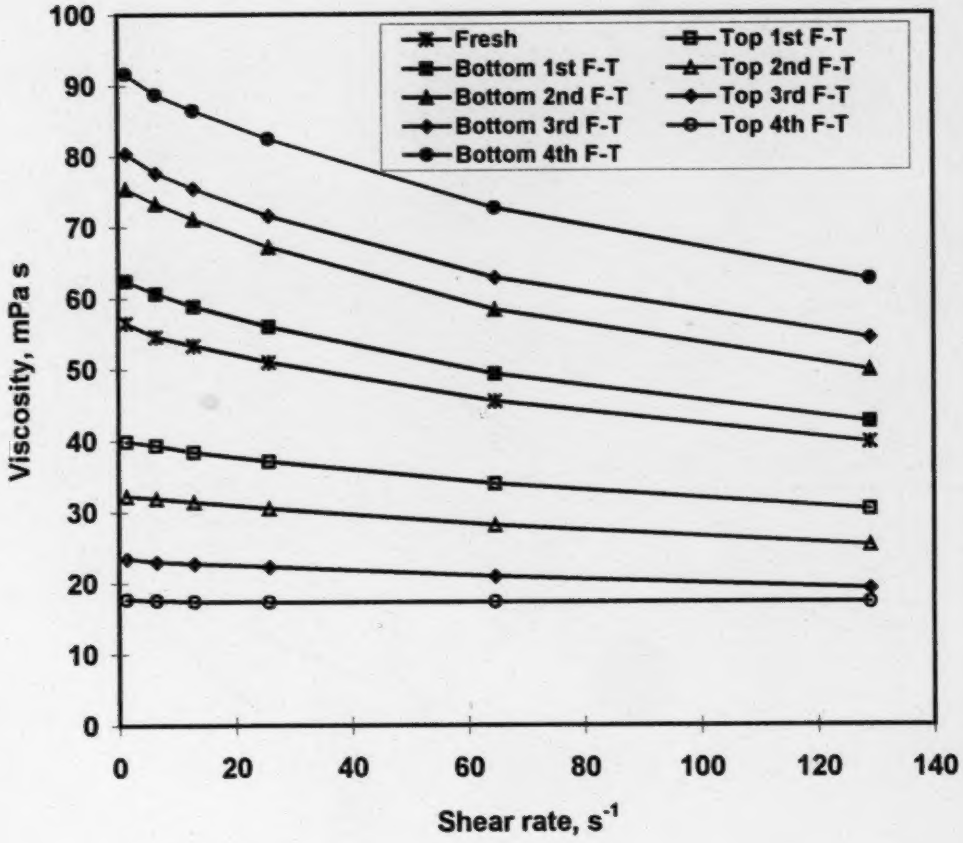

Figure 5.6. Viscosity change in top (open symbols) and bottom (solid symbols) layers of 0.5% (w/w) LAB gum after 4 freeze-thaw (F-T) cycles.

Surprisingly, 0.25% LAB gum solution lost only about 10% of its viscosity in the upper layer and gained approximately the same percentage in the bottom layer after the first F-T cycle (Fig. 5.7). The higher dilution probably protected the solution against forced agglomeration upon freezing. Cryogenic freezing may be expected to have a similar effect. After four F-T cycles, the top and bottom layers lost and gained approximately 35% in viscosity, respectively. No settling was visible at the end of the fourth F-T cycle, possibly due to the lower gum concentration. Reheating of the sample returned solution viscosity to that of the fresh solution.

Simulated "accidental" freezing (i.e. only one F-T cycle) followed by refrigerated storage showed an increase in the viscosity of the upper layer upon refrigerated storage of both the 1% PP and LAB solutions (Table 5.2). Even though, initially, it seemed to be a kind of viscosity recovery, after comparing the data with the changes in the viscosity of the control sample over time, it could be concluded that the viscosity increase indicates network formation and its strengthening over time.

5.3.4. Effect of long term refrigerated storage

Long term storage at 4°C showed an increase in the viscosity of fresh 1% PP and LAB gum solutions that did not undergo any freeze-thawing (Table 5.3). After 1 week, viscosity of PP gum increased from 18.2 to 18.9 mPa s, which indicated network formation throughout the solution. The yield point of fresh solution was non existent, while the 7 day-old solution exhibited a yield stress of 0.05 Pa, which increased to 0.07 Pa after 4 weeks. It is interesting that the maximum viscosity plateau of 18.9 mPa s was reached after few consecutive measurements, which indicates that sample stress may trigger and/or speed up β-glucan association. Such a possibility was also described by

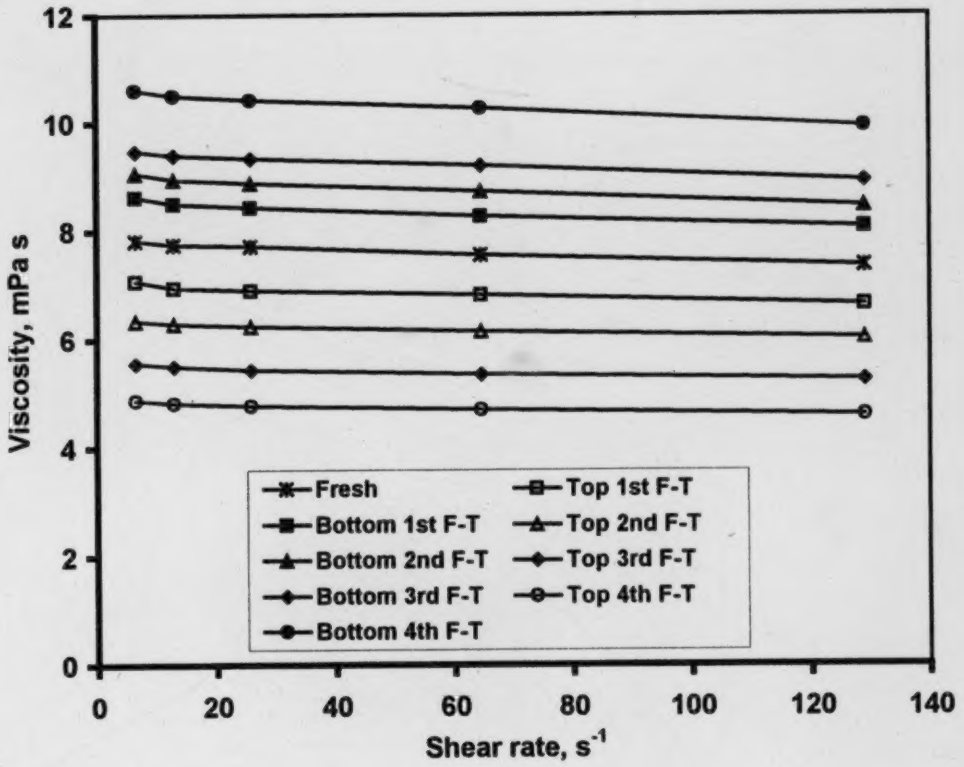

Figure 5.7. Viscosity change in top (open symbols) and bottom (solid symbols) layers of 0.25% (w/w) LAB gum after 4 freeze-thaw (F-T) cycles.

TABLE 5.2

Changes in the Apparent Viscosity of the Top Layer of 1% (w/w) PP and LAB Solutions After One Freeze-Thaw (F-T) Cycle and Subsequent Refrigerated Storage. Viscosity Measured at 10 rpm (12.9 s^{-1}) and 20°C.

	Viscosity, mPa s				
Gum	**Fresh**	**1st F-T**	**2nd Day**	**3rd Day**	**4th Day**
PP	18.2	8.47	9.13	9.77	10.3
LAB	693	492	506	545	587

TABLE 5.3

Changes in the Apparent Viscosity of 1% (w/w) PP Disturbed and Not Disturbed Solutions During Storage and 1% (w/w) LAB Gum Solution Over 4-Week Storage at 4°C. Viscosity Measured at 10 rpm (12.9 s^{-1}) and 20°C.

	Viscosity, mPa s				
Gum	Fresh	1st Week	2nd Week	3rd Week	4th Week
PP dist.	18.2a	18.3-18.9[1]b	18.5b	18.8-19.1[1]c	20.3-21.3[1]d
PP not dist.	19.8	19.8-19.9	19.7-21.3	20.4-21.3	21.5-22.2
LAB	693a	702b	724c	725c	745d

[1] Increase in viscosity after few subsequent measurements. Average calculated for statistical differences.

a - d: Means with the same letter within a row are not significantly different ($p>0.05$).

Linemann and Kruger (1997). A longer storage time increased both the viscosity and pseudoplasticity of PP gum solution reaching 21.3 mPa s at 10 rpm after 4 weeks (a 17% increase). The flow behavior index decreased from 0.9940 to 0.9234 depicting an increase in pseudoplasticity (Table 5.4). It is interesting that after one week there was no change in pseudoplasticity, but a slight increase in viscosity, which may be the consequence of agglomeration-flocculation in the beginning, leading to a subsequently more substantial network formation after 2 weeks.

A second set of samples (Table 5.3), which was not disturbed during storage, underwent similar changes in viscosity with apparent agglomeration and network formation over time. The viscosity of disturbed samples was slightly lower than that of the corresponding undisturbed samples, again demonstrating the importance of sample history.

Fresh LAB gum solution also exhibited a significant ($p \leq 0.05$) viscosity increase over 4 weeks of storage (Table 5.3). The apparent yield stress of fresh solution was 0.01 Pa. The origin of this stress cannot be ascribed to network formation, but rather to a kind of flocculation similar to the case of emulsion stabilization by xanthan gum (Parker et al. 1995). After one week of storage, viscosity increased by about 1%, while yield stress was still at the level of 0.01 Pa. A longer holding period resulted in a viscosity increase of 7.5% by the end of 4 weeks testing time, while pseudoplasticity was changed only slightly (Table 5.4). A small increase in viscosity (approximately 1-2%) may be ascribed to evaporation (visible condensation inside tubes), while the rest is due to slow aggregation of β-glucan molecules. Yield point also increased to 0.02 Pa. It is important to note that refrigeration temperature slows down association of β-glucan (Burkus and Temelli 1999)

TABLE 5.4

Power Law Constants for 1% PP Gum Solution Over 4 Week Storage at 4°C. Viscosity Curves Measured at 20°C in the Range of 1-20 rpm (1.29-26.9 s^{-1}) for PP Gum Solutions and 1-100 rpm (1.29-129 s^{-1}) for LAB Gum Solutions.

Storage Time, Weeks	Consistency Coefficient, c	Flow Behavior Index, n	R^2
PP			
0	0.0185	0.9940	1
1	0.0192	0.9941	0.9999
2	0.0194	0.9821	0.9999
3	0.0202	0.9746	1
4	0.0260	0.9237	1
LAB			
0	1.2305	0.7263	0.9881
1	1.2446	0.7270	0.9883
2	1.2905	0.7243	0.988
3	1.2926	0.7238	0.9878
4	1.3328	0.7216	0.9877

and that registered changes would be more pronounced upon room temperature storage.

5.3.5. Scanning electron microscopy

SEM images of LAB and PP gum solutions are shown in Figures 5.8-5.13 at 1000x to 100,000x magnification. No special molecular structure was discernible even at 100K magnification. Freezing the sample in liquid nitrogen resulted in the formation of ice crystals, which sublimed during sample preparation and were seen as black regions or void spaces in the micrographs. Thus, the micrographs may reveal some information about BBG solution behavior. Both LAB and PP gum solutions at 1% concentration formed thin veil-like forms upon freezing in liquid nitrogen (Figs. 5.8 and 5.9, respectively). This veil was apparently more dense for the LAB solutions, which may be a consequence of its higher MW. At 0.5% concentration, the LAB network was more open because of more void space in the less dense solution (Fig. 5.10). Pilot Plant gum at 0.5% (not shown) exhibited a similar trend. The elongated parallel strands observed with fresh solution (Fig. 5.9) were probably due to freezing of the sample in liquid N_2 prior to SEM analysis. SEM micrographs of the 1% PP solutions that were subjected to a freeze-thaw cycle (Fig. 5.11) showed a more organized structure of β-glucan compressed into strands of fiber-like network. These strands had a lot of interconnections, resembling a honeycomb and they were much thicker than the veil-like structure of fresh solution. Bigger void spaces (Figure 5.11) were probably the result of sample inhomogeneity due to sample settling after a F-T cycle, as described previously.

A more concentrated, 3% PP solution (Fig. 5.12) that underwent gelation over a week of refrigerated storage exhibited nodes that are interconnected into a mesh-like network. That mesh may be able to retard crystal formation and growth in frozen or ice-

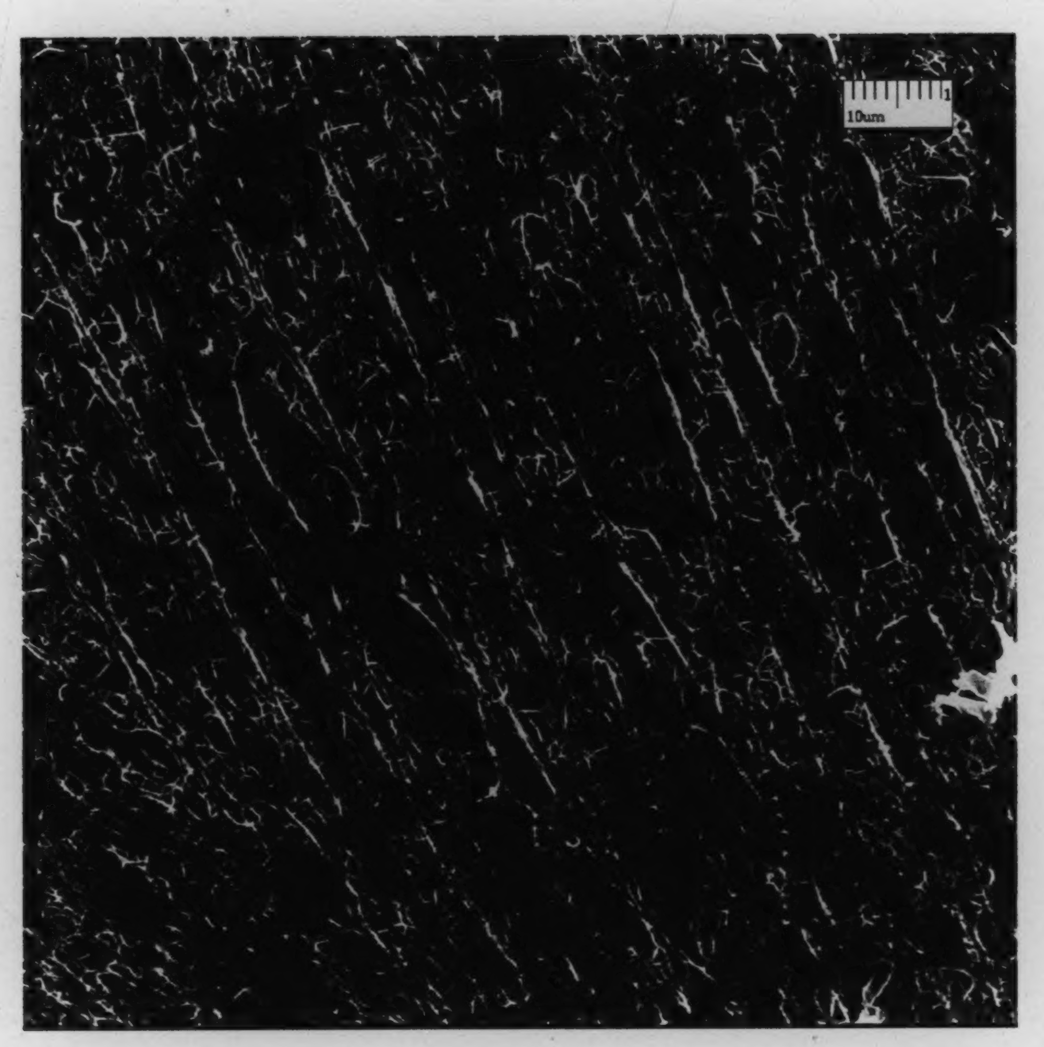

Figure 5.8. Scanning electron micrograph of 1% (w/w) fresh LAB solution (×1000).

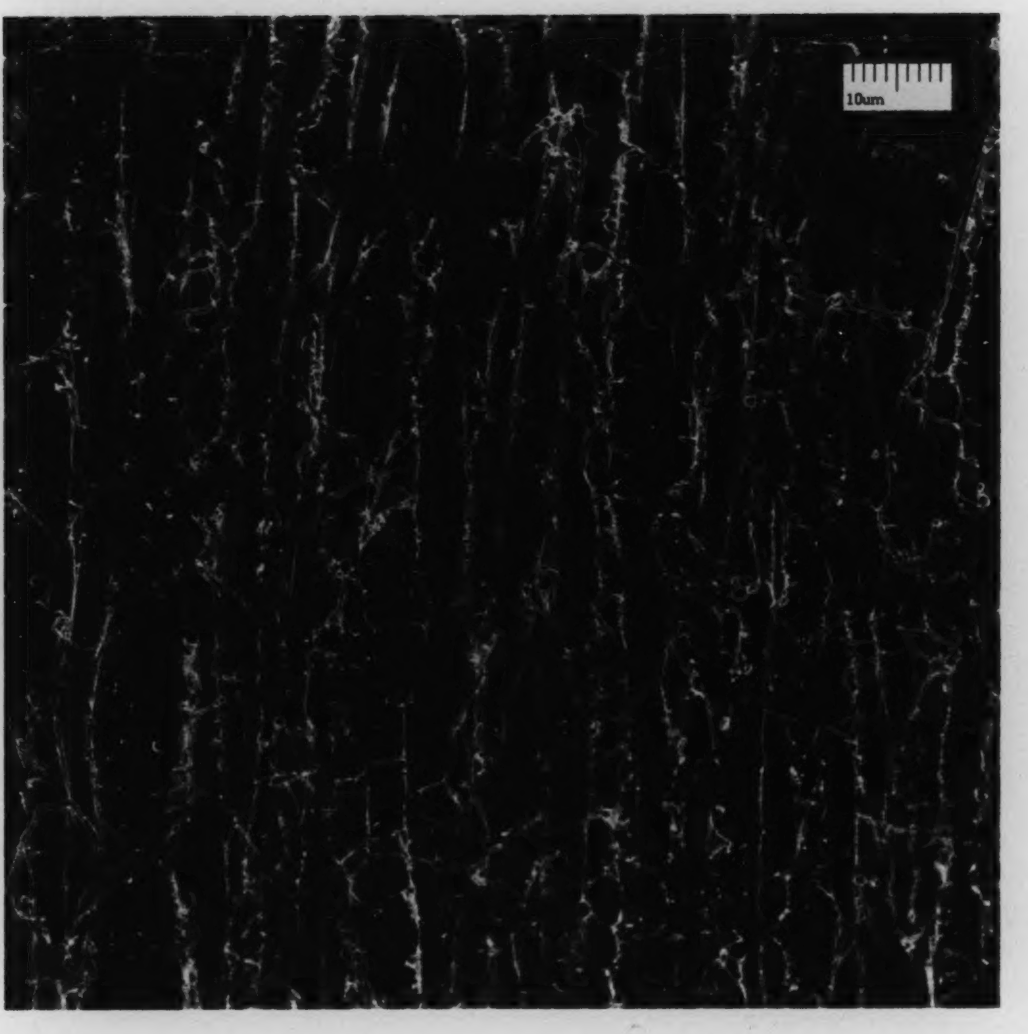

Figure 5.9. Scanning electron micrograph of 1% (w/w) fresh PP solution (×1000).

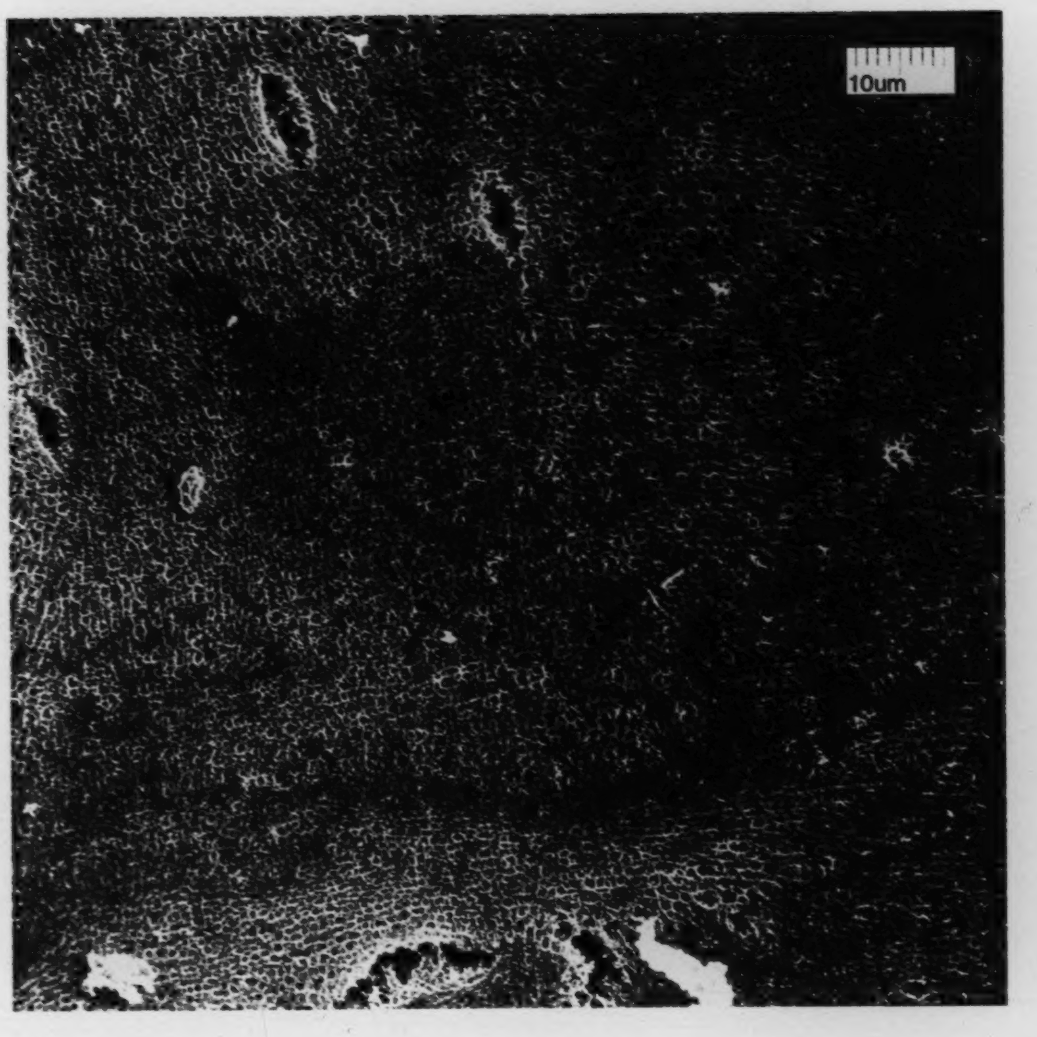

Figure 5.10. Scanning electron micrograph of 0.5% (w/w) fresh LAB solution (×1000).

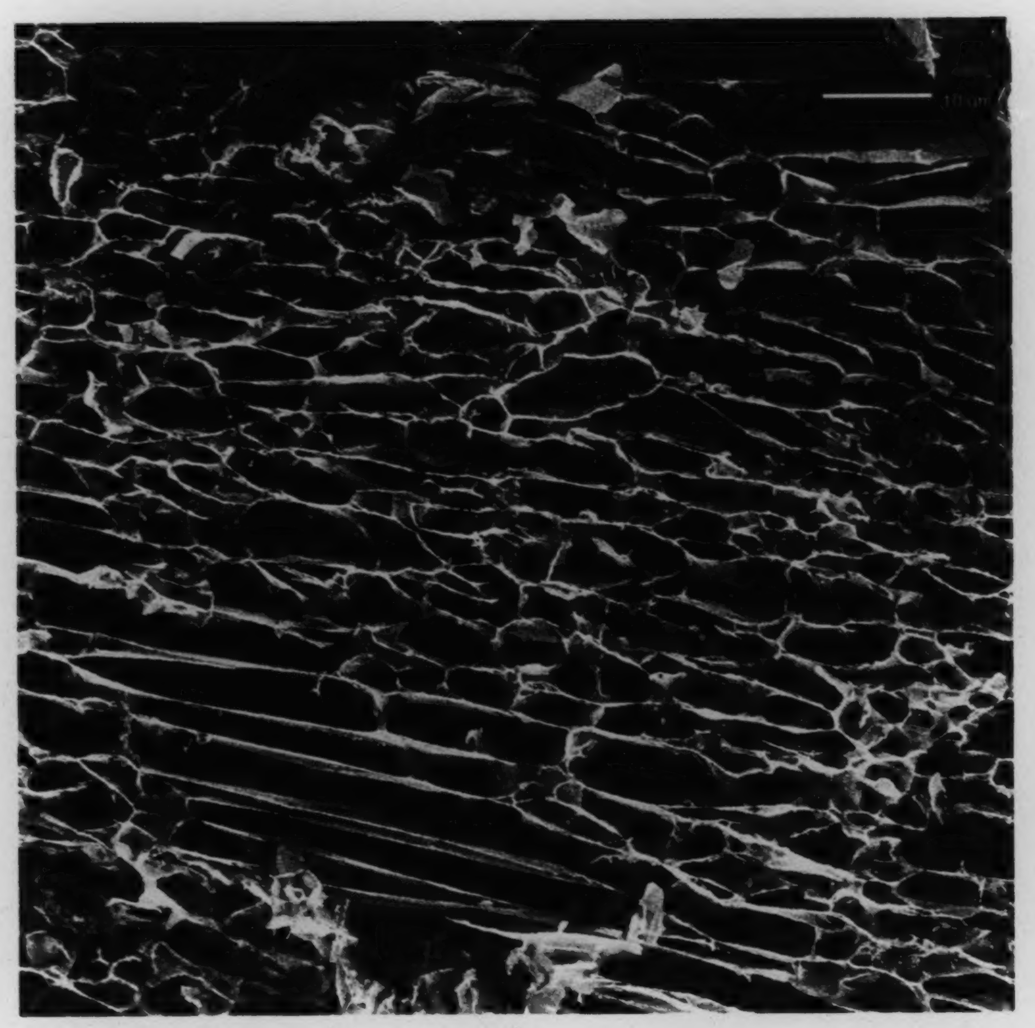

Figure 5.11. Scanning electron micrograph of 1% (w/w) PP solution after one F-T cycle (×1000).

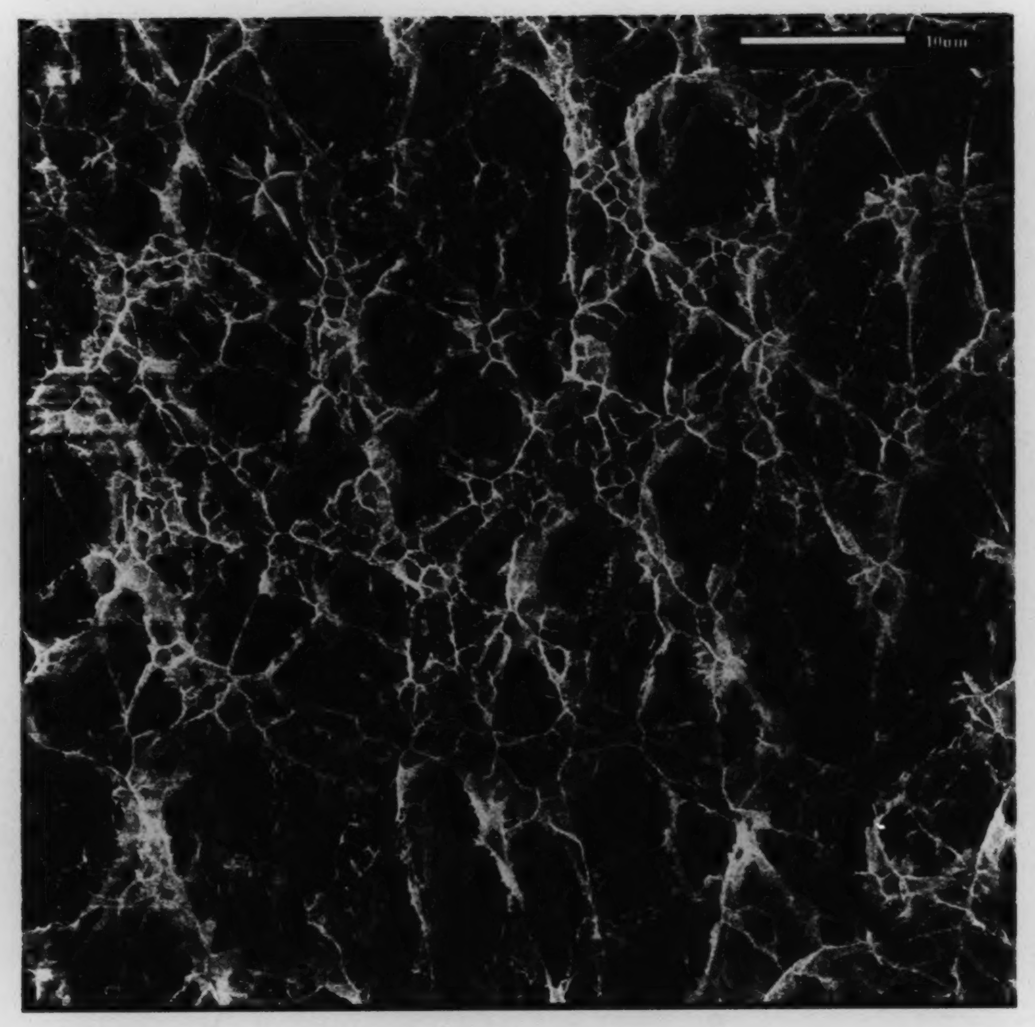

Figure 5.12. Scanning electron micrograph of 3% (w/w) PP solution after setting a network in a refrigerator for a week (×1500).

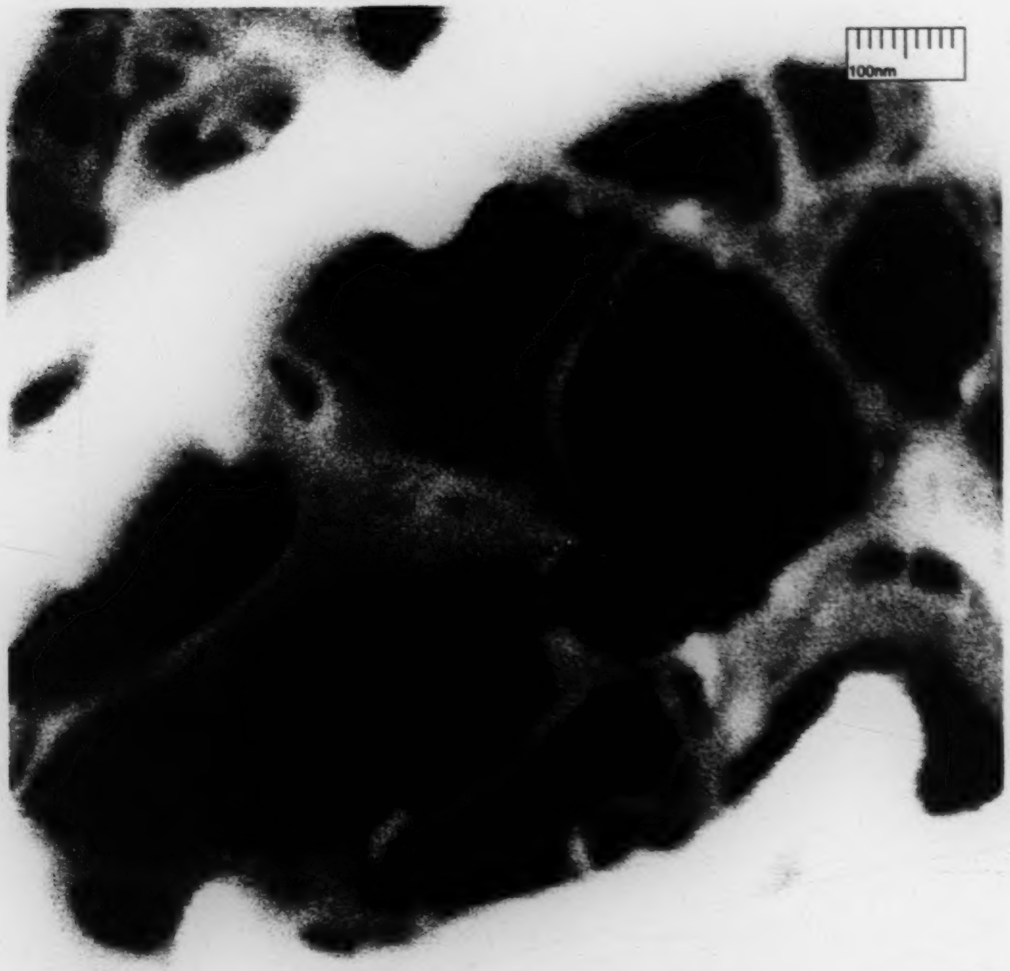

Figure 5.13. Scanning electron micrograph of 0.5% (w/w) fresh PP solution under higher magnification (×100,000).

cream type products, and further physically stabilize emulsions and foams. The mesh-like network formed over time in LV BBG solutions (and to a much lower extent in HV solutions) probably resembles the one formed in 0.5% PP solution upon freezing in liquid N_2 (Fig. 5.13, 100K magnification). Solutions at 0.25% concentration did not have a structure firm enough to support themselves and collapsed after sublimation and gold sputtering.

The image of the PP gum gel (Fig. 5.12) supports the idea of micelles and micellar flocs interacting with each other to form a three-dimensional network, as discussed earlier. Flocs may serve as centers for further aggregation-gelation when they become the new enlarged centers or nodes of the gel network structure. The new gel network nodes may look similar to the white regions associated with a higher concentration of β-glucan strands (Figure 5.12).

5.4. CONCLUSIONS

Surplus water used in the preparation of gum solutions had no significant influence ($p>0.05$) on the hydration of 1% PP and LAB gum. As well, the speed of cooling of the solutions following hydration did not have a significant influence ($p>0.05$) on the viscosity of either LAB or PP solutions.

The effect of Polytron shear on HV gum was proportional to the viscosity of solutions, which lost 15-50% viscosity in the 0.25-0.7% concentration range. Pilot Plant gum was completely resistant to Polytron treatment at the applied speed. It was also more resistant to ultrasonication, losing 10-12% of its viscosity, while LAB gum solutions lost 40-50% of their original viscosity. The higher resistance of the LV PP gum to shear is probably due to inherent differences in the inner micellar structure, which warrants further investigation.

Freezing lowered viscosity of the top PP solution layer by about 85% after four freeze-thaw cycles, causing visible gel-like settling after the second F-T cycle. On the other hand, 1% LAB gum was more resistant to freeze-thaw treatment, although showing a big drop in the viscosity of the top layer after the first freeze-thaw cycle. However, that change is not expected to be detrimental in food systems like ice cream because a network can be formed around food particles and stabilize them further, but changes in texture (i.e. chewiness) need to be evaluated. Reheating the samples following four F-T cycles resulted in complete recovery of the lost viscosity for both PP and LAB gum solutions, confirming that observed changes were due to reversible network associations.

Upon long term refrigerated storage, 1% PP solution viscosity increased steadily, probably due to β-glucan association. There was also an increase in pseudoplasticity despite its low viscosity (18-21 mPa s range) signaling network formation. The yield point, which was not detectable for the fresh 1% PP solution, reached 0.07 Pa after four weeks of storage at 4°C. There was a 7.5% viscosity increase for the 1% LAB solution during the four weeks period, with a slight increase in yield stress (from 0.01 to 0.02 Pa). Differences in the structure of the three-dimensional network formed upon freezing and refrigerated storage were demonstrated using scanning electron microscopy.

5.5. REFERENCES

Burkus, Z., and Temelli, F. 1998. Effect of extraction conditions on yield, composition and viscosity stability of barley β-glucan gum. Cereal Chem. 75:805-809.

Burkus, Z., and Temelli, F. 1999. Gelation of barley β-glucan concentrate. J. Food Sci. 64:198-201.

Dawkins, N. L., and Nnanna, I. A. 1995. Studies on oat gum [(1→3, 1→4)-β-D-glucan]: composition, molecular weight estimation and rheological properties. Food Hydrocol. 9:1-7.

Gaosong, J., and Vasanthan, T. 2000. Effect of extrusion cooking on the primary structure and water solubility of β-glucans from regular and waxy barley. Cereal Chem. 77:396-400.

Glicksman, M. 1969. Gum Technology in the Food Industry. Academic Press: New York.

Grimm, A., Kruger, E., and Burchard W. 1995. Solution properties of β-D-(1,3)(1,4)-glucan isolated from beer. Carbohydr. Polym. 27:205-214.

Harrington, R. E., and Zimm, B. H. 1965. Degradation of polymers by controlled hydrodynamic shear. J. Phys. Chem. 69:161-175.

Linemann, A., and Kruger, E. 1997. Relationships between structure and characteristics of β-glucan in beer production. Part 1: Properties in dissolving β-glucan out of beer. Brauwelt Int. 1997(IV):344-351.

Luchsinger, W. W., and Stone, B. A. 1976. Linkage sequencing of oligosaccharides by their rates of alkaline degradation. Carbohydr. Res. 46:1-8.

Morgan, K. R., and Ofman, D. J. 1998. Glucagel, a gelling β-glucan from barley. Cereal Chem. 75:879-881.

Morris, E.R. 1989. Polysaccharide solution properties: origin, rheological characterization and implications for food systems. Pages 132-163 in: Frontiers in Carbohydrate Research-1: Food Applications. R. P. Millane, J. N. BeMiller, and R. Chandasekaran, eds, Elsevier Applied Science. New York, N.Y.

Parker, A., Gunning, P. A., Ng, K., and Robins, M. M. 1995. How does xanthan stabilize salad dressing? Food Hydrocol. 9:334-332.

SAS Institute Inc. 1989. SAS/STAT User's Guide, Version 6, Fourth Edition Vol. 2, Cary, NC.

Szu, S. C., Zon, G., Schneerson, R., and Robbins, J. B. 1986. Ultrasonic irradiation of bacterial polysaccharides. Characterization of depolymerized products and some applications of the process. Carbohydr. Res. 152:7-20.

Ulrich, S. E., Clancy J. A., Eslick, R. F., and Lance, R. C. M. 1986. β-Glucan content and viscosity of extracts from barley. J. Cereal Sci. 4:279-285.

Varum, K. M., Smidsrod, O., and Brant, D. A. 1992. Light scattering reveals micelle-like aggregation in the (1→3),(1→4)-β-D-glucan from oat aleurone. Food Hydrocol. 5:497-511.

White, C. A., and Kennedy, J. F. 1988. Identification and structural analysis of monomeric and polymeric carbohydrates. Pages 42-72 in Carbohydrate Chemistry. J. F. Kennedy, ed., Oxford University Press: New York, NY.

Wood, P. J., Weisz, J., Fedec, P., and Burrows, V. D. 1989. Large scale preparation and properties of oat fractions enriched in (1→3)(1→4)-β-D-glucan. Cereal Chem. 66:97-103.

Wood, P. J., Weisz, J., and Blackwell, B. A. 1991a. Molecular characterization of cereal β-D-glucans. Structural analysis of oat β-D-glucans and rapid structural evaluation of β-D-glucans from different sources by high-performance liquid chromatography of oligosaccharides released by lichenase. Cereal Chem. 68:31-39.

Wood, P. J., Weisz, J., and Mahn, W. 1991b. Molecular characterization of cereal β-glucans II, size exclusion chromatography for comparison of molecular weight. Cereal Chem. 68:530-536.

6. THE INFLUENCE OF pH AND TEMPERATURE ON THE VISCOSITY OF BARLEY β-GLUCAN[1]

6.1. INTRODUCTION

The non-ionic nature of the β-glucan polymer gives it stability and constant viscosity in the pH range 2-10 (Dawkins and Nnanna 1995). Dawkins and Nnanna (1995) tested the effect of temperature on the viscosity of oat β-glucan (OBG) at neutral pH and found it to be stable after heating to 100°C. Burkus (1996) observed decreased viscosity of barley β-glucan (BBG) gum after 30 min extraction at 60°C and pH 10. Initial pH, adjusted with Na_2CO_3, was approximately 10.3 in order to compensate for buffering during extraction. Extraction at pH 9 at the laboratory scale resulted in high viscosity (HV) gum. However, the combination of high pH and shear was detrimental for the viscosity of pilot plant BBG gum (Chapter 2).

Knuckles and Chiu (1999) were able to obtain HV BBG during extraction at pH 9 and 100°C. BBG degradation occurred after treatment with strong acids (0.1*N* HCl and 5% trichloroacetic acid). The molecular weight (MW) of wheat β-glucan extracted by Cui et al (1999) at 60°C with the help of a strong base, 0.1-1 *M* NaOH, was lower than that of samples extracted at room temperature. Beer et al (1997) noticed decreased MW of both BBG and OBG after extraction with 5:0.5 (%) $NaOH:NaBH_4$ mix for 16 h at room temperature (22°C). A similar observation was discussed by Bhatty (1999) with respect to his extraction with 4% NaOH at room temperature for 1 h (Bhatty 1995). Wood et al (1991) registered a drop in MW of oat and barley β-glucan extracted at 80°C using pH 10 Na_2CO_3 buffer. Lower MW was also registered in the bran sample after extraction at 60°C as opposed to 45°C. Wood et al (1989) had previously speculated about the detrimental influence of plant polyphenols on the MW of OBG, and a similar high pH-polyphenols combination could be partially responsible for β-glucan cleavage in the bran sample tested by Wood et al (1991).

---

[1] A version of this chapter is to be submitted to Cereal Chemistry for consideration for publication.

Doublier and Wood (1995) intentionally hydrolyzed OBG using 0.1*N* HCl at 70°C for 15 or 60 min and obtained a less viscous product having 30 % and 8% of its original viscosity, respectively, despite an increase in purity from 81% to 89% β-glucan. Bansema (2000) measured a significant drop in the viscosity of pH 3 BBG solution after 30 min treatment at 55, 75 or 95°C. Results for pH 5 and 9 were less conclusive. However, no change in the viscosity of a β-glucan rich beverage was observed after 8 weeks of storage at 5°C, although the pH was in the 2.6-3.3 range (Bansema 2000).

A review of the above studies leads to a few general conclusions about the viscosity stability of β-glucan solutions at different pH conditions. Strong bases can influence MW of β-glucan even at room temperature and over relatively short periods of time (about 1 hr). β-Glucan seems to be unaffected at pH 9, while its stability at pH 10 is temperature dependent. Strong acidic conditions such as 0.1*N* HCl at elevated temperatures, as well as a pH of about 3, can also cleave β-glucan. However, long term stability of β-glucan at different pH conditions is not known and requires further investigation. A better understanding of β-glucan viscosity stability is critical for optimization of extraction processes as well as for β-glucan product applications.

Since the BBG used throughout this thesis work was extracted at 55°C and stabilized at 90°C (Chapter 2), the goal of this study was to verify the viscosity stability of BBG obtained at pilot plant (PP) and laboratory (LAB) scale at these two processing temperatures and different pH environments. As well, long term stability over several weeks at critical pH was examined.

6.2. MATERIALS AND METHODS

6.2.1. Materials

Pilot Plant and LAB gums were produced as described in Chapter 2 according to Burkus and Temelli (1998). Sodium carbonate, HCl and standard pH 2-10 buffers were obtained from Fisher Scientific (Nepean, ON). The common milk preservative Brotab 10 (6 mg of Bronopol and 0.3 mg of Natamyicin per 20 mg tablet; Systems Plus, New Hamburg, ON) was used at the level of one tablet per 25 g solution. Pure HV barley β-glucan (>99% purity, viscosity of 1% (w/v) solution specified by the manufacturer as 114 cSt and MW of 327,000) was purchased from Megazyme International Ireland Ltd. (Bray, Ireland). Distilled water was from the local supply.

6.2.2. High-temperature tests at pH 2-10

BBG solutions of PP and LAB gums were prepared in desired concentration, 0.5-2% (w/w), as previously described by Burkus and Temelli (1998). High temperature tests at pH 2-10 were performed by mixing equal amounts of gum solution and a buffer so that the final concentration of PP gum was 1% and that of LAB gum was 0.5%. The pH of samples was adjusted, as necessary, using small aliquots of Na_2CO_3 in powder form or concentrated HCl in order to avoid dilution of samples. After high temperature treatment in an oven at 55 and 90°C for 60 min (it took approximately an additional 5 min to reach the desired temperature), samples in capped vials were cooled in an ice water bath with gentle stirring, their weights adjusted to compensate for evaporation, and stirred on a magnetic stirrer for 1 min to homogenize samples and then taken for viscosity measurements.

Consecutive 4x1 h heating periods at 90°C and pH 9 were undertaken to verify the influence of the pH at which these gums were stabilized during the extraction procedure in the pilot plant. An additional LAB sample at pH 9 (actual pH=9.04) was prepared using sodium carbonate to investigate possible ionic effects on hydrolysis. Blank samples prepared with distilled water were subjected to the same procedure to distinguish the influences of pH from those of possible thermal or chemical degradation of 1.0% PP, 0.5% LAB and 0.5% pure BBG gum solutions.

6.2.3. Long term storage tests

Long term tests were carried out using 1% PP and 0.5% LAB gum solutions over a four-week period at pH 2, 3, 4 and 9 at room temperature (~22°C). Samples were prepared as described previously and approximately 10 g was placed into capped vials so that, technically, the whole sample was used for viscosity tests. The same sample was used for repeated tests over four weeks. No preservative was used in the first set of samples on the assumption that the low pH would prevent microbial growth. However, PP solutions at pH 2 were infected with mold and some of the LAB solutions at pH 2 and 3 also showed mold growth. Therefore, Brotab 10 was used in repeated tests. Viscosity was measured every 7 days and compared with that of control samples at day zero. To verify the influence of pH, at the end of the four-week period samples, were neutralized, reheated at 90°C for 30 min to melt any network structures or agglomerates that may have formed and viscosity was measured again.

6.2.4. Viscosity measurements

Viscosity was determined by consecutive fixed speed tests using a PAAR Physica UDS 200 rheometer (Glenn Allen, VA) equipped with a Peltier heating system. Tests were performed at 20±0.03°C using the DG 27 cup and bob geometry with double gap and a 7 mL sample size. Sample size was not measured by volume due to difficulties in measuring highly viscous samples. Rather, the clean DG 27 cup was placed on the balance, tared and 7.01 ± 0.005 g of sample was weighed directly into the cup. Shear rate data are reported as rpm or, after multiplication by the conversion factor 1.29, as s^{-1}.

6.2.5. Statistical analysis

Each solution at every pH was prepared in duplicate. Viscosity measurements were carried out in triplicate for each sample. Analysis of variance of the results was performed using the General Linear Model procedure of SAS Statistical Software, Version 6 (SAS Institute, 1989). Multiple comparison of the means was performed using the least significant difference (LSD) test at $\alpha=0.05$ level.

6.3. RESULTS AND DISCUSSION

6.3.1. High temperature tests at pH 2-10

High temperature test results for PP and LAB gum solutions are shown in Tables 6.1 and 6.2, respectively. After mixing of the samples with buffer solutions, an interesting phenomenon was observed. Pilot Plant solutions at pH 8-10 and LAB solutions at pH 9 and 10 had viscosities higher than those of their neutral counterparts. Viscosity increased with pH. It seems that the high pH buffer environment causes either swelling of β-glucan micelles or their agglomeration into larger flocks. After adjusting the pH to 7 by adding

TABLE 6.1
Changes in the Apparent Viscosity of 1% (w/w) PP Gum Solutions at 10 rpm and pH 2-10 After 1hr Thermal Treatment at 55 and 90°C.

		Viscosity, mPa s		
pH Buffer	**Actual pH**	**Fresh**	**55°C**	**90°C**
Control[1]	6.96	18.8a	18.8a	18.6a
2	2.75	18.1a	18.0a	14.0b
3	3.25	18.6a	18.6a	16.6b
4	4.27	18.6a	18.5a	18.0b
5	5.16	18.5a	18.5a	18.3a
6	6.15	18.3a	18.2a	18.3a
7	7.01	18.5a	18.4a	18.2a
8	7.67	19.8a	19.9a	19.4a
9	8.84	20.9a	20.7a	20.5a
10	9.94	23.1a	23.0a	16.1b

[1] Mixed with distilled water.

a, b: Means with the same letter within a row are not significantly different (p>0.05).

TABLE 6.2
Changes in the Apparent Viscosity of 0.5% (w/w) LAB Gum Solutions at 10 rpm and pH 2-10 After 1hr Thermal Treatment at 55 and 90°C.

		Viscosity, mPa s		
pH Buffer	**Actual pH**	**Fresh**	**55°C**	**90°C**
Control[1]	6.50	56.2a	55.1b	53.3c
2	2.40	53.1a	51.2b	7.9c
3	3.12	55.8a	55.0a	25.1c
4	4.13	56.2a	55.9a	51.8b
5	5.06	55.8a	55.3a	53.2b
6	6.05	54.0a	53.2b	53.1b
7	6.96	56.3a	56.1a	54.5b
8	7.71	56.1a	56.2a	55.9a
9	8.81	63.0a	62.6a	56.6b
10	9.92	79.6a	79.1a	28.7b

[1] Mixed with distilled water.

a-c: Means with the same letter within a row are not significantly different ($p>0.05$).

<10 µL of concentrated HCl, viscosity decreased to that of neutral solutions. This finding confirmed that the phenomenon is pH related but did not explain the mechanism. Changing the pH of neutral solutions using sodium carbonate or sodium and potassium phosphates in different combinations did not yield any increase in viscosity even after 1 h holding time or after heating to 55°C. Understanding the mechanism of this phenomenon warrants further investigation.

Heating at 55°C for 1 h, as expected, was less detrimental to the viscosity of both PP and LAB gum solutions than heating to 90°C (Tables 6.1 and 6.2, respectively). The viscosity of LAB and PP gum solutions was stable at each pH, with the exception of LAB gum in pH 2 buffer, and even that drop of about 3.6% from the viscosity of the unheated sample should not be considered as technologically significant since there is no industrial process that may last one hour at 55°C and pH 2. Surprisingly, neither pH 9 nor pH 10 buffers caused a viscosity change at 55°C.

Heating at 90°C for 1 h caused significant ($p \leq 0.05$) changes in viscosity of both PP and LAB solutions at extreme pH (Tables 6.1 and 6.2). In the pH 2 buffer, LAB gum lost approximately 80% of its original viscosity while PP gum lost only 23% of its original viscosity. Such a difference may be in part due to the actual pH being lower in the LAB solution, 2.4 vs. 2.76 in PP pH 2 buffer solution. At pH 3, PP gum lost 11% of its original viscosity, while at pH 4 viscosity drop was only 3.2%. Similarly, LAB gum lost 55% and 8% of its original viscosity at pH 3 and 4, respectively. Slightly lower actual pH values of LAB gum solutions would not be sufficient to cause such a big difference in viscosity change, but a plausible explanation may be that the $(1\rightarrow3)$-β bond is more sensitive to low pH and some of these bonds were already degraded during the production of PP gum. As well, more compact PP gum micelles (Chapter 5) may be more resistant to acid hydrolysis.

Laboratory gum underwent apparently significant ($p \leq 0.05$) viscosity changes at pH 5, 6 and 7 after 1 h heating at 90°C. However, the drop in viscosity was not higher than that in the control sample with distilled water. Consequently, the change in viscosity was not influenced by pH conditions, but by some kind of heat related structural change or degradation with an unknown mechanism.

Despite the statistical significance of the viscosity drop at low pH and 90°C, it is not anticipated that any industrial process would last more than 1 min at 90°C and pH 3 and 4. Therefore, no substantial technological consequences due to these changes are anticipated. However, changes observed in alkali conditions would have implications for β-glucan extraction processes.

Treatment at pH 9 caused only a 3% drop in the viscosity of the PP sample, but there was a 30% loss in viscosity at pH 10. Laboratory gum solutions lost 13% and 64% of their original viscosity at pH 9 and 10, respectively. Despite a significant ($p \leq 0.05$) viscosity loss for LAB gum solutions at pH 9, viscosity was still above the range of 53-56 mPa s for samples at pH 5-8. Therefore, additional tests were undertaken and the pH of apparently hydrolyzed samples was neutralized. None of the pH 2, 3, 4, 9 and 10 samples treated at 90°C could recover its original viscosity, confirming that viscosity loss is due to hydrolysis and not due to some kind of conformational change.

Consecutive 4x1 h heating at 90°C of both PP and LAB solutions in pH 9 buffer is shown in Figure 6.1. Laboratory gum solutions at pH 9 were not stable, losing 7.6% of their original viscosity after the first hour, and about 16% after the fourth hour of treatment.

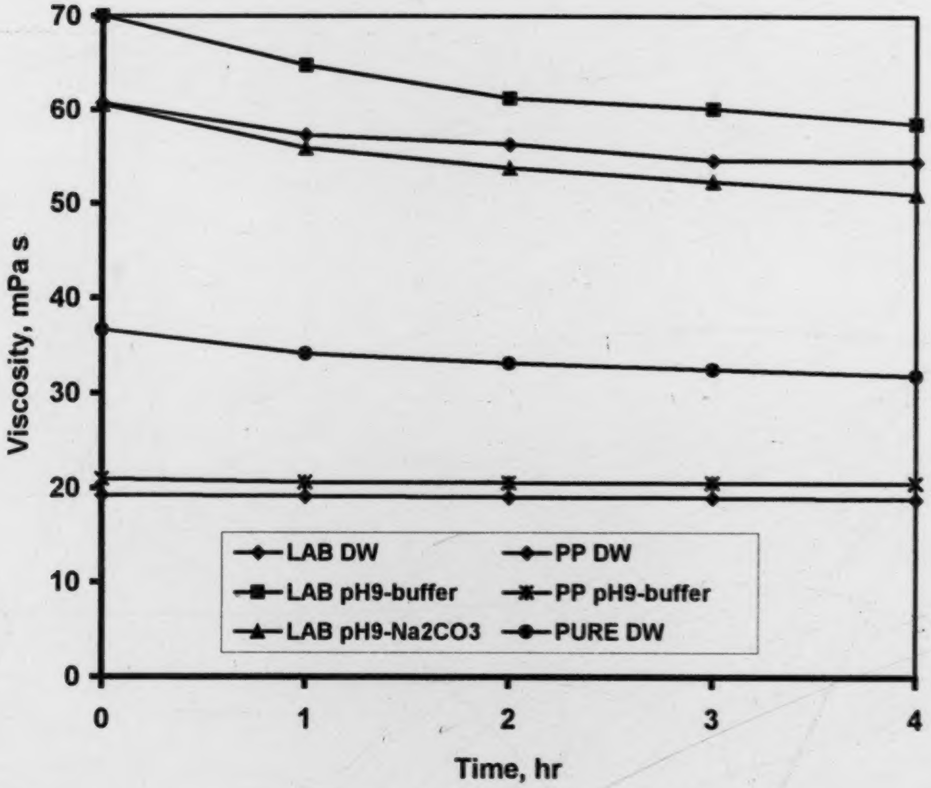

Figure 6.1. Viscosity change in 0.5% (w/w) LAB, 0.5% (w/w) Pure BBG and 1% (w/w) PP solutions after 4 × 1 h consecutive heat treatments at 90°C. Solutions prepared with distilled water (DW), pH 9 buffer or sodium carbonate (coefficient of variation for each data point <2.5%).

LAB gum solution prepared with buffer was neutralized after the fourth hour of heating and its viscosity dropped to the level of the sample prepared with sodium carbonate. Interestingly, LAB sample prepared with distilled water, which served as a control, also lost some of its original viscosity, but its viscosity leveled off after the third hour of treatment, while the pH 9 samples continued to lose about 2.6-3% of their viscosity in the fourth hour. It seems that BBG solutions in both cases, distilled water and high pH environment, parallely underwent some conformational change during heating and cooling, and only about 5% of the total viscosity loss after 4 h of heating (calculated as the difference in the viscosity loss of the pH 9 sample and the control sample prepared with distilled water) could be ascribed to high pH conditions. However, this change cannot be limited to high pH alone since a combined effect of high pH and polyphenol remnants in the LAB gum cannot be *a priori* excluded.

Pilot Plant solutions were more resistant to high pH than LAB gum solutions and viscosity loss was only 0.4-0.5 mPa s in the samples prepared with distilled water and pH 9 buffer (Fig. 6.1). Again, such a small viscosity loss is probably due to either the influence of some other component(s), such as polyphenolics, or a kind of thermal degradation with unknown mechanism, but not due to high pH itself.

A small viscosity loss due to high pH in LAB solution and no loss in PP solution may be the consequence of the higher sensitivity of the (1→3) bond to high pH conditions (McCleary and Codd 1991). Having fewer of these bonds in the low viscosity PP gum results in the higher stability of this gum in a high pH environment. Extended holding of the PP extract at pH 9 during the production of PP gum (Chapter 2) would have been responsible for a small part of the viscosity loss, while the high shear applied during the separation of phases would be the major reason for the viscosity loss observed during pilot plant production of BBG gum (Chapter 5) .

Pure barley BBG gum prepared at 0.5% concentration and subjected to the same 4x1 h treatment as LAB gum also lost 13% if its original viscosity, from 36.6 to 31.8 mPa s (Fig. 6.1). Similar to LAB gum, the main viscosity loss of about 6.9% was after the first 1 h treatment, with a tendency to level off afterwards.

6.3.2. Long term storage tests

Long term storage tests (Table 6.3) carried out at different pH levels displayed interesting behavior of PP gum solution. After week 1, there was no significant change ($p>0.05$) in the viscosity of pH 2, 3 and 4 PP gum solutions, while after week 2 the increase in viscosity was significant ($p\leq0.05$) with a corresponding increase in the pseudoplasticity of samples (Table 6.4). The trend was more pronounced after week 3 showing much higher pseudoplasticity and apparent viscosity. It must be mentioned that the samples were disturbed during pipetting into measuring cup and while bringing the bob into measuring position so that the reported changes underestimate the real change in an undisturbed system.

After four weeks, the trend continued and viscosity as well as pseudoplasticity increased further (Tables 6.3, 6.4 and Fig. 6.2). Solutions were apparently more turbid and gel-like particles were visible on the vial walls when solutions were in a thin layer.

Pilot Plant gum at pH 9 exhibited similar behavior, but in somewhat delayed fashion. There was no significant change ($p>0.05$) in the viscosity of pH 9 PP solution after the first 2 weeks, but pseudoplasticity was already higher. At week 3, both viscosity and pseudoplasticity increased significantly ($p\leq0.05$), while at 4 weeks the sample

TABLE 6.3
Changes in the Apparent Viscosity of 1% PP and 0.5% LAB Solutions Measured at 10 rpm (12.9 s^{-1}) and 20°C During 4-Week Storage at Room Temperature and Different pH

		Viscosity, mPa s				
Gum	**pH Buffer**	**Fresh**	**Week 1**	**Week 2**	**Week 3**	**Week 4**
PP	2	18.8a	18.6a	19.5b	20.9c	25.0d
	3	18.7a	18.7a	19.9b	34.1c	54.5d
	4	18.9a	18.9a	19.8b	34.5c	59.3d
	9	20.2a	20.1a	20.3a	28.7b	ph. sep.[1]
LAB	2	59.1e	53.0d	49.4c	45.6b	42.1a
	3	61.5c	60.2c	59.1b	58.4ab	57.8a
	4	61.6b	60.5a	60.3a	59.5a	60.0a
	9	70.2a	71.6a	71.1a	70.5a	71.1a

[1] Phase separation occurred.

a-d: Means with the same letter within a row are not significantly different (p>0.05).

TABLE 6.4
Power Law Flow Behavior Index (n) for 1% PP and 0.5% LAB Gum Solutions at Different pH During 4 Week Storage at Room Temperature. Viscosity Curves Measured in the Range 1-20 rpm (1.29-26.9s^{-1}) at 20°C.

	Power Law Flow Behavior Index, n				
pH buffer	**Fresh**	**Week 1**	**Week 2**	**Week 3**	**Week 4**
PP					
2	0.9929	0.9919	0.9672	0.9312	0.8285
3	0.9939	0.9937	0.9566	0.7365	0.6427
4	0.9932	0.9926	0.9614	0.6894	0.5543
9	0.9906	0.9911	0.9784	0.8665	ph. sep.
LAB					
2	0.9675	0.9711	0.9764	0.9798	0.9806
3	0.9636	0.9666	0.9685	0.9682	0.9691
4	0.9630	0.9644	0.9649	0.9657	0.9651
9	0.9601	0.9584	0.9596	0.9599	0.9582

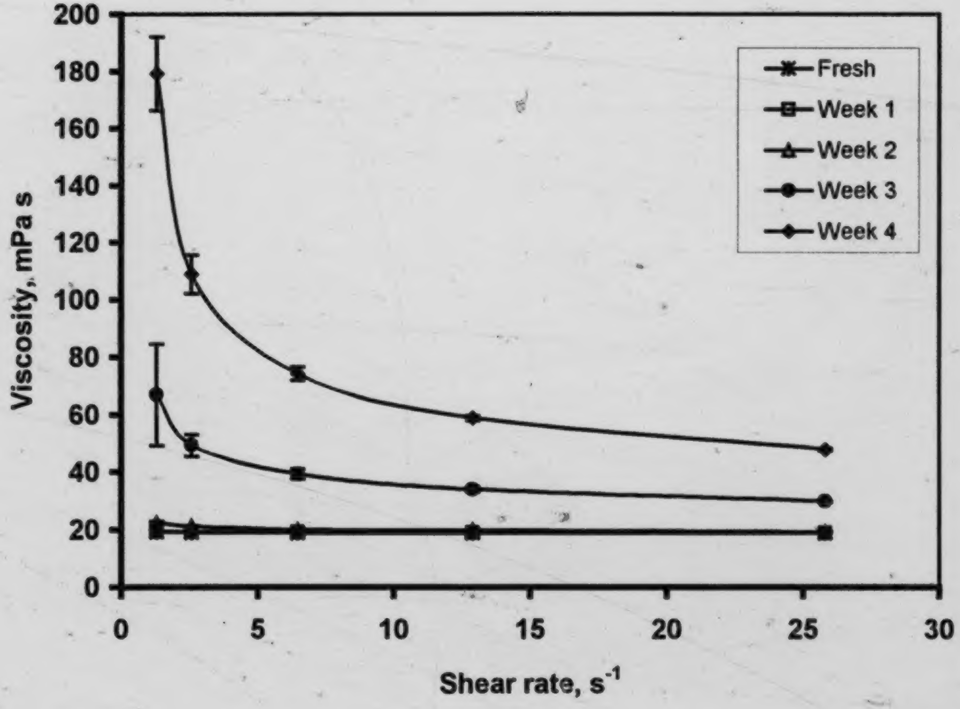

Figure 6.2. Viscosity change of 1% (w/w) PP gum solutions prepared with pH 4 buffer during 4 week storage at room temperature.

underwent phase separation and viscosity was not measured. Clear upper and turbid lower gel-like layers were sharply separated.

Reheating after neutralization of solutions initially at pH 2, 3, 4 and 9 did not result in any change in viscosity from the original at time zero. This finding confirmed that all rheological changes discussed above are due to agglomeration and network formation.

Laboratory gum lost about 30% of its viscosity over the 4 week storage period at pH 2 with a slight decrease in pseudoplasticity, while at pH 3 viscosity loss was only 6% and cannot be ascribed solely to low pH since the effectiveness of the preservative could have been somewhat compromised over the long holding period at room temperature. At pH 4 and 9, no significant changes ($p>0.05$) in viscosity due to pH alone were observed throughout the 4 week period.

6.4. CONCLUSIONS

Thermal treatment of 55°C for 1 h in pH 2 buffer, was detrimental to the viscosity of both PP and LAB gum solutions. In buffers with pH ≥3, viscosity changes, although statistically significant ($p\leq0.05$), do not represent a technological problem since, in practice, thermal treatments are, in general, rather short.

At 90°C, holding at pH 9 for 1 h caused a minor drop in viscosity of LAB gum. Most of the viscosity loss was attributed to factors other than pH. Pilot Plant gum was more stable at pH 9 and 90°C, hinting at possible minor structural differences between HV and LV gums. High pH (>9) may degrade β-glucan when accompanied by temperatures >55°C. Consecutive heating (4x1 hr) at pH 9 confirmed the higher sensitivity of HV LAB gum to higher temperatures at high pH, but also demonstrated that thermal degradation is happening regardless of environmental pH.

Long term storage (4 weeks) proved to be detrimental to the stability of a 1% PP solution regardless of pH. The innate tendency to gel creates a network which, if disturbed too much, undergoes phase separation when the solution is actually a weak gel. Without any disturbance, the solution will form a weak gel after prolonged holding. Therefore, at the given concentration of 1%, application of LV PP BBG gum is limited.

LAB gum did not undergo network formation at low pH over the 4 week period. Viscosity loss was pronounced only in the pH 2 buffer and stability at pH>3 confirms the possibility of successful food application regardless of concentration.

6.5. REFERENCES

Bansema, C. 2000. Development of a Barley β-Glucan Beverage With and Without Whey Protein Isolate. M. Sc. Thesis. University of Alberta, Edmonton, AB.

Beer, M. U., Wood, P. J., and Weisz, J. 1997. Molecular weight distribution and (1→3)(1→4)-β-D-glucan content of consecutive extracts of various oat and barley cultivars. Cereal Chem. 74:476-480.

Bhatty, R. S. 1995. Laboratory and pilot plant extraction and purification of β-glucans from hull-less barley and oat bran. J. Cereal Sci. 22:163-170.

Bhatty, R. S. 1999. The potential of hull-less barley. Cereal Chem.76:589-599.

Burkus, Z. 1996. Barley β-glucan: Extraction, Functional Properties and Interaction with Food Components. M. Sc. Thesis. University of Alberta, Edmonton, AB.

Burkus, Z., and Temelli, F. 1998. Effect of extraction conditions on yield, composition and viscosity stability of barley β-glucan gum. Cereal Chem. 75:805-809.

Cui, W., Wood, P. J., Weisz, J., and Beer, M. U. 1999. Nonstarch polysaccharides from preprocessed wheat bran: Carbohydrate and novel rheological properties. Cereal Chem. 76:129-133.

Dawkins, N. L., and Nnanna, I. A. 1995. Studies on oat gum [(1→3, 1→4)-β-D-glucan]: composition, molecular weight estimation and rheological properties. Food Hydrocol. 9:1-7.

Doublier, J. L., and Wood, P. J. 1995. Rheological properties of aqueous solutions of (1→3) (1→4)-β-D-glucan from oats (*Avena sativa* L.). Cereal Chem. 72:335-340.

Knuckles, B. E., and Chiu, M. M. 1999. β-Glucanase activity and molecular weight of β-glucans in barley after various treatments. Cereal Chem. 76:92-95.

McCleary, B. V., and Codd, R. 1991. Measurement of (1→3),(1→4)-β-D-glucan in barley and oats: a streamlined enzymic procedure. J. Sci. Food Agric. 55:303-312.

SAS Institute Inc. 1989. SAS/STAT User's Guide, Version 6, Fourth Edition Vol. 2, Cary, NC.

Wood, P. J., Weisz, J., Fedec, P., and Burrows, V. D. 1989. Large scale preparation and properties of oat fractions enriched in (1→3)(1→4)-β-D-glucan. Cereal Chem. 66:97-103.

Wood, P. J., Weisz, J., and Mahn, W. 1991. Molecular characterization of cereal β-glucans II, size exclusion chromatography for comparison of molecular weight. Cereal Chem. 68:530-536.

7. STABILIZATION OF EMULSIONS AND FOAMS USING BARLEY β-GLUCAN[1]

7.1. INTRODUCTION

(1→3)(1→4)-β-D-Glucan exhibits health benefits such as lowering of blood cholesterol (Newman et al 1992, Kahlon et al 1993, Pick 1994), regulating blood glucose level and insulin response in diabetics (Wood 1993, Pick 1994, Gosain 1996) and even antitumor activity (Eastwood 1987). Oats and barley are the richest commercial natural sources of β-glucan with levels usually in the range of 4-7%. Enrichment from these sources can be achieved by extraction using a neutral or an alkaline medium to obtain a gum containing 45-81% (dm basis) β-glucan (Wood et al 1989, Bhatty 1995, Burkus and Temelli 1998), while Morgan and Ofman (1998) using hot-water extraction followed by filtering and two freeze-thaw cycles could achieve >90% purity. In order to incorporate barley β-glucan (BBG) gum into value-added food products, information about its functional properties is essential.

Medium- (MV, 20-100 mPa s) and high-viscosity (HV, >100 mPa s) BBG gum solutions exhibit pseudoplastic behavior at 0.5% and 1% concentration levels (Bhatty 1995, Burkus and Temelli 1998), while HV gum at low concentrations (≤0.25%) or low-viscosity (LV, <20 mPa s) gum solutions (≤1%) are Newtonian (Burkus and Temelli 1998). At shear rates below 10 s^{-1}, pseudoplasticity of HV oat β-glucan could be observed even at a concentration of 0.1% (Dawkins and Nnanna 1995).

At room temperature, oat β-glucan solutions were stable at pH 2-10 (Dawkins and Nnanna 1995) and BBG was stable at pH 2-11 (Grimm et al 1995), but solution stability was not tested as a function of time. The very high extraction pH of 1 *N* NaOH solution resulted in lower viscosity of oat β-glucan (Bhatty 1995). Burkus and Temelli (1998) found that a high pH-high temperature combination (pH 10/45°C) during extraction had a detrimental impact on the viscosity of a β-glucan solution. This may be due to the sensitivity of the (1→3)-β-D bond to high pH (McCleary and Codd 1991). Different pH-temperature-time combinations applied during processing may affect β-glucan viscosity and decrease the stability of food systems such as salad dressings if β-glucan is applied as a stabilizer.

---

[1] A version of this chapter is published in Food Research International (33:27-33, 2000).

The presence of other food ingredients can affect the properties of hydrocolloids. Sucrose, at concentrations of 20-50%, increased viscosity of oat and barley β-glucan, while higher concentrations of 65 or 75% lowered viscosity (Autio 1987, 1988, Dawkins and Nnanna 1995, Bansema 2000). Conflicting results have been reported on the effect of salt on the viscosity of β-glucan. Autio et al (1987) found no salt influence on viscosity in the concentration range 1-10%, whereas Dawkins and Nnanna (1985) observed a pronounced viscosity drop at higher NaCl concentrations. Burkus (1996) indicated that the order of hydration plays a role in such cases and β-glucan should be dissolved first to achieve higher viscosity. BBG gum shows strong incompatibility with milk, whey, whey protein concentrate (WPC) and starch at certain concentrations (Burkus 1996) and its application in fluid systems such as beverages may result in separation of phases (Bansema 2000).

Gelation of β-glucan extracted from barley was confirmed by Burkus and Temelli (1999) and Morgan and Ofman (1998). Formation of a soft gel on cooling was observed by Morgan and Ofman (1998) when the concentration of BBG was >0.5%. This was a clear indication of the existence of a yield point in BBG solutions. Aggregation may be responsible for the thixotropic behavior of β-glucan extracted from beer (Linemann and

Kruger 1997). Thixotropy disappeared at 55°C due to the "collapse of the structure", which may be better described as melting or dissociation of micelles. Data reported by Linemann and Kruger (1997) describe the existence of a yield stress which, besides increasing viscosity, may have a role in the stabilization of food systems.

Hydrocolloids are added to foams and emulsions in order to increase the viscosity of the continuous phase, thus decreasing bubble and droplet movement, slowing down drainage and creaming, and resulting in more stable foams and emulsions. According to Stoke's law, separation of the dispersed phase is proportional to the density difference between the phases and the square of the droplet (bubble) diameter, but inversely proportional to the viscosity of the continuous phase. In emulsions, an increase in the viscosity of the continuous phase to close to or greater than the viscosity of the dispersed phase facilitates drop breakage and emulsion formation (Gaonkar 1991). Gelling hydrocolloids form a network around the drops, adding to stability. Charged hydrocolloids have the ability to lower interfacial tension, adding to stability as well (Gaonkar 1991). BBG is a non-ionic polysaccharide. Therefore, it is expected to facilitate stabilization by increasing viscosity and, probably, by forming a weak network. Such physical properties of BBG have not been evaluated. Temelli (1997) prepared foams which were stable for 2 h. LV BBG gum alone was used at a level of 2.5%. However, stabilization of foams and emulsions using higher viscosity β-glucan at lower concentrations (≤1%) and in the presence of another stabilizer such as WPC has not been reported.

The objectives of this study were to investigate the physical properties of BBG, such as surface activity and differential scanning calorimetry (DSC) behavior, to examine if BBG gum can stabilize foams and emulsions alone and in the presence of WPC, to determine the influence of sugar on foam stability, and to study the effect of BBG viscosity (LV or HV) on the stability of foams and emulsions.

7.2. MATERIALS AND METHODS

7.2.1.Materials

BBG gums were produced at the pilot plant (PP) and laboratory (LAB) scale as described in Chapter 2 according to Burkus and Temelli (1998). Pilot Plant gum was of LV type, whereas LAB gum was of HV type.

Pure barley β-glucan (>99% purity) of low- (LV) and high-viscosity (HV) (viscosity of 1% (w/v) solutions specified by the manufacturer as 5.9 and 114 cSt and MW of 137,000 and 327,000, respectively) were purchased from Megazyme International Ireland Ltd. (Bray, Ireland).

WPCs, Alacen 841, Alacen 878 (New Zealand Milk Products, Santa Rosa, CA) and RT-80 extra grade (DMV, La Crosse, WI) were pretested for foam volume and time for 50% drainage after beating for 5 min using a Black & Decker PowerPro MX50W kitchen mixer equipped with whisks (Black & Decker Canada Inc., Brockville, ON) at maximum speed. Alacen 841 was selected for further testing since its drainage time was several times longer than that of the others. The composition of Alacen 841 was (as is basis): moisture 4.4%, protein (N x 6.38) 79.3%, ash 3.0%, fat 6.5%, lactose 5.5%. Food grade canola oil and sugar were purchased at a local grocery store.

7.2.2. Sample Preparation

BBG solutions (1%, w/w) were prepared according to Burkus and Temelli (1998). WPC powder (1% w/w for emulsions, 5% w/w for foams) was added into BBG solutions and stirred for 1 h at room temperature for complete hydration. After mixing with oil, the WPC concentration was 0.5%, a typical usage level for emulsifiers. WPC level of 5% for foams was selected based on Phillips et al (1987).

Emulsions (25 mL, 50% oil-in-water) were prepared in duplicate in graduated tubes using a Polytron homogenizer (model PT-2000, Kinematica AG, Littau, Switzerland) equipped with a 20 mm toothed generator at speed 1 for 2 min. The aqueous phase was BBG solution and/or WPC. The emulsion created with WPC served as a control, while the addition of PP and LAB gums were the treatments studied. For easier evaluation of emulsions, the aqueous phase was colored with 100 μL of blue "Food Club" color (Scott-Bathgate Ltd., Winnipeg, MB), while the color of the oil was enhanced with 200 mg/100 mL of crystalline β-carotene (Nutritional Biochemicals Corporation, Cleveland, OH). Even though the oil content of a typical emulsion product (e.g. mayonnaise) is >75% (v/v), a level of 50% was used in this study to allow creaming measurements in a reasonable time frame and since BBG shows potential as a fat replacer (Burkus and Temelli 1998).

Foams were prepared in duplicate using WPC as the control. The addition of PP and LAB BBG gums and sugar (10%, w/w) were the treatments studied. Sugar was added to BBG + WPC solutions and stirred for 10 min prior to whipping with a kitchen mixer (Black & Decker PowerPro MX50W) for 10 min at maximum speed using whisks and a stainless steel bowl with straight sides. Speed was increased gradually during the first minute.

7.2.3. Physical Properties Determination

Surface and interfacial tension measurements were performed on BBG and WPC solutions (0.5% and 1%) in air and canola oil, respectively, using the Axisymmetric Drop

Shape Analysis-Profile technique (ADSA-P) described by Li et al (1992). ADSA-P is a digital image processing technique which measures pendent (or sessile) drop shape: curvature radius, drop volume, drop surface area and contact angle. It also takes into account local gravity and densities of the measured liquid and surrounding fluid. The apparatus consisted of a light source, light diffuser, video camera with microscope, digitizer, monitor and computer. A syringe was used to create a drop hanging at the tip of a teflon tube (KF22TF teflon needle, Chromatographic Specialties Inc., Brockville, ON). The whole system except the monitor and computer was placed on a pneumatic table to minimize vibration. An image of a calibration grid on an optical glass was recorded for both calibration and correction of the optical distortion. Coordinates of a hanging string were taken to correct for the camera angle. Surface tension was calculated on the basis of the recorded image using the same software used by Li et al (1992). Calculations were repeated 15 times for each drop image and an average value was presented. Measurements were performed in duplicate.

The surface tension for distilled water was determined to be 72.2 mJ/m^2 at 23°C and 50% relative humidity, which compares well with the literature value of 72.3 mJ/m^2 (CRC 1988). However, for time sequence measurements, surface tension and interfacial tension measurements had to be carried out in a covered cuvette to prevent evaporation from the drop surface and to hold the oil, respectively. In this case, optical distortion was high and surface tension values were about 1.6 units lower than those measured in air. Since it was impossible to measure a blank for interfacial tension without optical distortion due to the cuvette, values obtained for water vs. canola oil were adjusted according to those reported by Gaonkar (1989).

Differential Scanning Calorimetry (DSC) was performed in duplicate on approximately 5% and 3% BBG suspensions at 5°C/min using a Du Pont 910 Differential Scanning Calorimeter coupled with a Du Pont 990 Thermal Analyzer (Du Pont Instruments, Wilmington, DE). BBG was weighed accurately into aluminum pans (Du Pont Instruments, 900796-901) and distilled water was added to bring the total weight to 25-30 mg. Sealed pans were equilibrated for 1 h at room temperature. Ice water, gallium and indium were used for calibration of the system. An empty pan was used as a reference.

Melting of BBG gel and reversibility of gelation were tested in capped glass tubes. BBG solutions at 5.5% concentration were prepared using LV-pure gum and LV Condor gum extracted from barley at pH 7 as described by Burkus and Temelli (1998). Gels were prepared as described by Burkus and Temelli (1999) and were allowed to set overnight at room temperature (~22°C). Tubes containing the gel were immersed in circulating water bath at 45°C and the temperature was increased in increments of 1°C/15 min. Melting of the gel was verified visually by tilting the tubes over 90° and checking for flow. Reversibility was tested by holding melted gels overnight at 40°C.

7.2.4. Stability of foams and emulsions

Emulsion stability was determined by measuring average oil droplet diameter (Northern Exposure image analysis, version 2.9h, Empix Imaging Inc., Mississauga, ON, equipped with a microscope and a camera) and creaming (visually) over a period of 3 weeks. Magnification was 2500x for emulsions prepared with BBG + WPC. The smallest discernible droplet size was approximately 0.7 µm. Error in droplet size determination was 0.13 µm or 1 pixel. Emulsions prepared with WPC or LAB gum alone were recorded at 250x magnification and had precision one order of magnitude lower than that for BBG +

WPC. For every emulsion, 2-3 pictures were taken at 0 and 21 days.

Viscosity of the aqueous phase of emulsions was determined in duplicate at 4 rpm using a Haake Rotoviscometer (model RV-3, Gebrueder HAAKE, Berlin, Germany). The viscometer was equipped with a MK 500 measuring head and a NV viscosity sensor system (8 mL cup) with a tempering vessel to maintain the temperature at 25±0.2°C.

Foam volume and time to 50% drainage were determined in graduated cylinders. Foam was transferred from the bowl by pouring gently down the walls of an inclined cylinder so as not to disturb the foam and to ensure that no air pockets were trapped in the cylinder.

7.2.5. Statistical Analysis

Foams and emulsions were prepared in duplicate. The physical properties of each foam and emulsion were also determined in duplicate except for 50% drainage measurements of foams. The viscosity of each sample was measured three times. Analysis of variance of the results was performed using the General Linear Model procedure of SAS Statistical Software, Version 6 (SAS Institute 1989). Multiple comparison of the means was performed by the least significant difference (LSD) test at $\alpha=0.05$ level.

7.3. RESULTS AND DISCUSSION

7.3.1. Physical Properties

Surface and interfacial tension

Addition of WPC to water instantly decreased the surface tension of water from 72.2 mJ/m^2 to 47.3 mJ/m^2, with a gradual further decrease to 45.1 mJ/m^2 in 8 min (Fig. 7.1). In a similar fashion, interfacial tension of water was reduced from 31.5 mJ/m^2 to 20.5

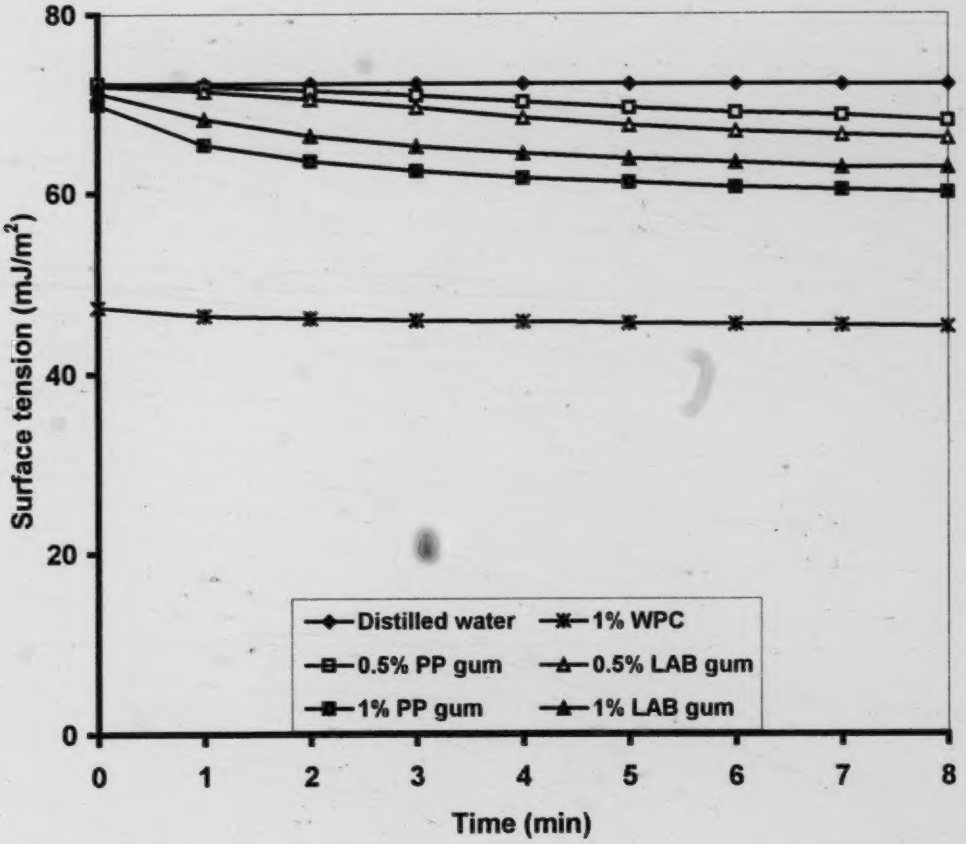

Figure 7.1. Surface tension of BBG gum and WPC solutions measured as a function of time.

mJ/m^2 at time 0, with a subsequent reduction to 19.8 mJ/m^2 after 8 min under the influence of WPC (Fig. 7.2).

Measurements carried out at the water-air and water-oil interface (Fig. 7.1 and 7.2, respectively) showed that BBG gum exhibits mild surface activity, probably due to the presence of impurities such as protein (1.4% in PP and 3.3% in LAB gum). Surface tension dropped <3 mJ/m^2 after the first min and <10 mJ/m^2 in 8 min for 1% gum solutions. For 0.5% solutions, the decrease in surface tension was <1mJ/m^2 after 1 min. A higher gum concentration resulted in increased surface activity, which was slowed by the high viscosity of LAB gum. A similar pattern was observed at the water-oil interface where the decrease in interfacial tension was 2-3 mJ/m^2 after 1 min and 4.7-6.3 mJ/m^2 after 8 min. With the ADSA-P technique, the surface tension of relatively large aqueous pendent drops (3-5 mm) was measured. Droplet size was bigger in oil where a smaller difference in specific gravity produces larger droplets. Surface active proteins move from inside a droplet to the outer layers and deposit onto the surface. This diffusion decreases with time. The small decrease in surface activity generated by BBG gum may contribute to foam and emulsion stabilization; however, it is not expected to be the dominant mechanism because of the greatly increased surface area of the numerous small droplets.

Differential scanning calorimetry

DSC measurements (Fig. 7.3) demonstrated that LV-pure BBG (~5%, w/w) had increased heat flow at 58-62°C, which implies the melting of a network. An enlarged DSC curve for LV-pure gum (Fig. 7.4) shows an endothermic peak in the region 58-62°C, which is in agreement with Morgan and Ofman (1999) who observed an endothermic peak at 58°C for their Glucagel extracted from barley. Enthalpy of melting (ΔH) was 0.3 kJ/kg.

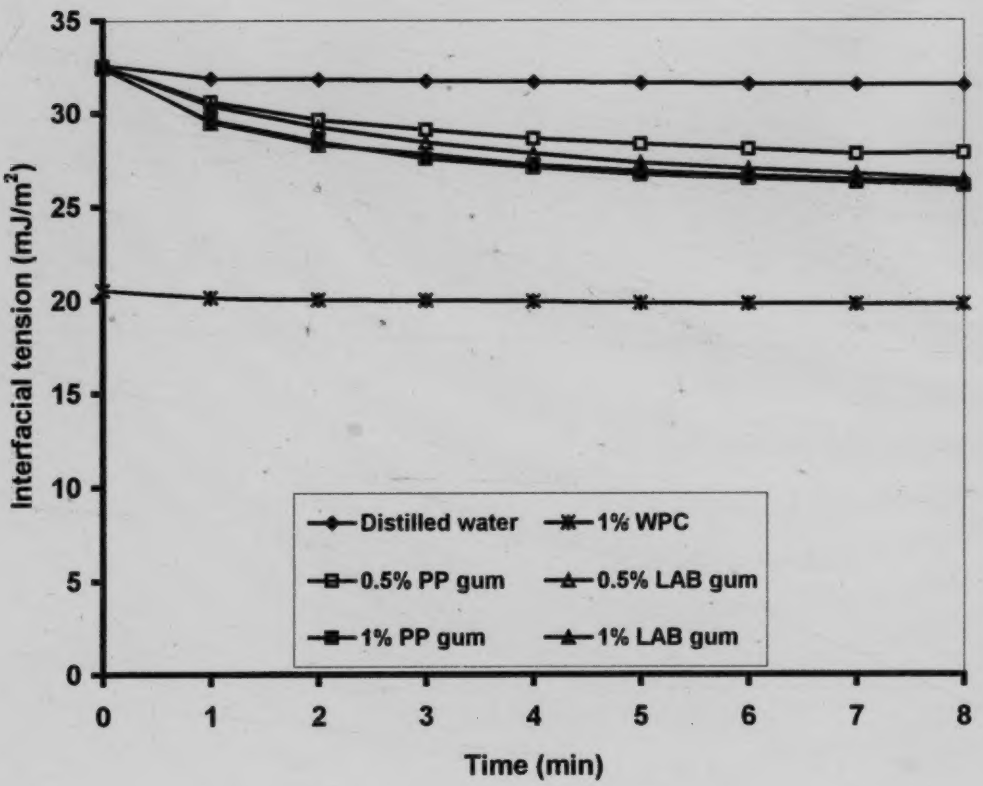

Figure 7.2. Interfacial tension of BBG gum and WPC solutions measured as a function of time.

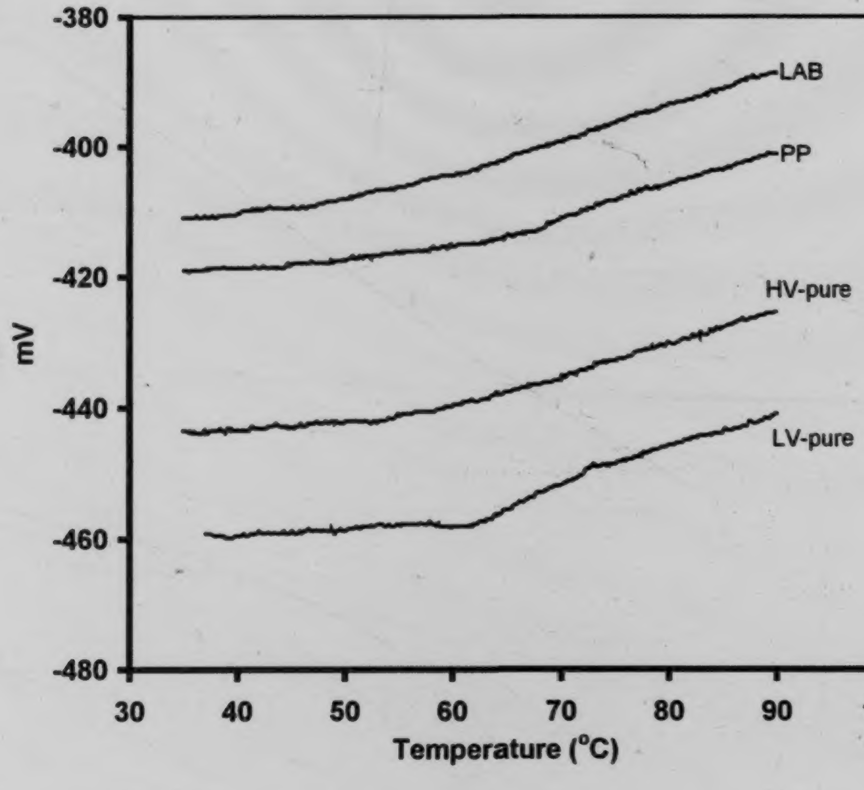

Figure 7.3. Differential scanning calorimetry at 5°C/min for different barley β-glucan gums at approximately 5% (w/w) concentration.

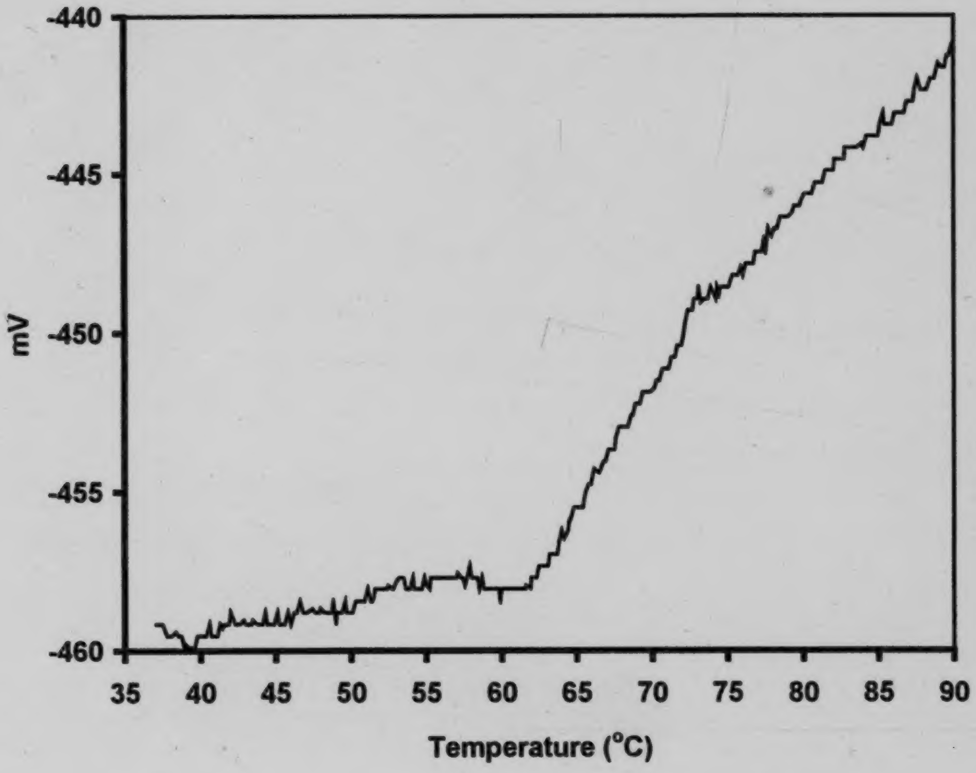

Figure 7.4. Enlarged DSC of approximately 5% (w/w) LV-pure BBG gum at 5°C/min.

HV-pure gum did not exhibit any endothermic peak. However, an inflection point was observed at approximately 58.3°C. The lack of a peak may be attributed to its high viscosity, which prevents formation of a network and crystallinity of network knots (Burkus and Temelli 1999). Compared to pure β-glucan, PP and LAB gums (approximately 5%) did not show any sharp change in the heating pattern, probably due to a less uniform MW distribution and the presence of impurities. However, the change in slope implies loosening of the internal structure, which allows higher heat conductivity.

A DSC pattern similar to those displayed in Figure 7.3 was recorded for 3% solutions, which did not gel in 24 h. This behavior implies a weak network formation even at low concentrations and the existence of yield stress, which is an additional stabilizing factor.

Gel melting and reversibility

In an effort to better understand the DSC behavior of BBG gums, melting of gels was evaluated visually with increasing temperature. Visible gel softening depicted as gel sagging started at approximately 59°C. The first signs of flow were present at 62°C and fluid flow commenced at 63°C, with a few chunks still present on the fluid surface, probably due to drying of gel surface despite capping the tubes. At 66°C, the solution was quite fluid with a small particle on the surface. Melting was complete at <70°C. If some shear was applied, melting would probably be completed sooner. The only shear applied in this experiment was tilting of tubes. Gel formed again when these tubes were allowed to sit overnight at 40°C. The reversibility of gels held at 20°C was verified previously (Burkus 1996). Repeated heating caused these gels to start flowing at 62°C, but with visible clumps present.

These observations correspond with the DSC results, indicating that melting of the gel network started at approximately 58°C with an expected temperature lag for visible effects at 59°C. Once network melting was completed or almost completed at ≥62°C, free flow could commence.

7.3.2. Stability of Foams and Emulsions

Emulsion stability

Emulsions prepared using PP gum alone (20.5 mPa s, 1%, w/w) exhibited rapid phase separation. Addition of WPC alone resulted in somewhat stable emulsions, but with relatively "large" droplets (24.7μm) (Table 7.1), which did not change significantly ($p>0.05$) in size over 21 days. Creaming of this emulsion was already quite pronounced after 24 h without a stabilizer to fill the spaces among droplets, and reached 33.2% after 21 days. High viscosity (683.0 mPa s) LAB gum alone could not stabilize emulsions at a 1% level. There was a significant ($p\leq0.05$) increase in droplet size from 53.3 μm to 67.5 μm after 21 days, and phase separation was enhanced with some free oil apparent on the surface.

The average droplet size of emulsions (3.6-3.8 μm) prepared with a mix of BBG gum and WPC (Table 7.1) increased to 4.3-5.0 μm after 21 days. However, this increase was not detrimental for stability since the average droplet size, although significantly increased ($p\leq0.05$), was still very small. WPC emulsions stabilized with PP gum had a larger increase in droplet size and substantial creaming (15.2%), while emulsions stabilized with LAB gum were less prone to coalescing and exhibited no creaming even after 21 days (Table 7.1). According to Stoke's law, increased viscosity is the main stabilizing factor in emulsions when droplet diameter is stable. An additional stabilizing factor is yield

TABLE 7.1
Droplet Size and Creaming of Emulsions Prepared with 1% (w/w) WPC and 1% (w/w) BBG Gum Prepared in the Laboratory (LAB) or Pilot Plant (PP) [a].

	Gum			
Parameter	WPC	LAB	PP + WPC	LAB + WPC
Droplet size, μm				
0 days	24.7b	53.3c	3.6a	3.8a
21 days	23.8c	67.5d	5.0b	4.3a
Creaming [b], %	33.2d	20.0c	15.2b	0.0a
Viscosity [c], mPa s	1.1a	683.0c	22.8b	690.2d

[a] Means with the same letter within a row are not significantly different ($p>0.05$).
[b] Vol. % of total emulsion volume of 25 mL after 21 days.
[c] Viscosity of the aqueous phase prior to emulsion preparation.

stress, which occurs in emulsions with high oil content even without a stabilizer (Wendin et al 1997). When oil content is lower, addition of a stabilizer enhances yield stress either by causing extensive flocculation (Cao et al 1990), or by the creation of a network and mechanical suspension of oil droplets or particles (Glicksman 1969).

Attempts to further characterize emulsions containing BBG were not successful. Trial rheological measurements (Rheometrics' RMS 800 rheometer) of emulsions demonstrated a breakdown in emulsion structure and apparent yield stress could be calculated by extrapolation, although with poor reproducibility. In addition, emulsions prepared without BBG gums could not be measured precisely. Determination of apparent yield stress by extrapolation can be misleading as in the case of xanthan gum when Parker et al (1995) proved that stabilization of salad dressing is a consequence of depletion flocculation and not of the creation of yield stress by xanthan itself. Depletion flocculation is also able to create an apparent yield stress by creating a weak particle gel network (Dickinson and Golding 1997). The behavior of BBG is expected to be similar to that of oat β-glucan examined by Doublier and Wood (1995), where hydrolyzed oat gum, resembling our PP gum, exhibited gel-like behavior, while high-viscosity unhydrolyzed oat gum, resembling our LAB gum, did not have such tendency. In such an evaluation, the time factor, responsible for subsequent network formation, should be taken into consideration.

Foam volume and stability

Volume and stability of foams (Figs. 7.5 and 7.6) were significantly ($p \leq 0.05$) increased when BBG gum was used as a stabilizer with LAB gum exhibiting significantly ($p \leq 0.05$) better stability than PP gum. Foams prepared with WPC alone at 5%

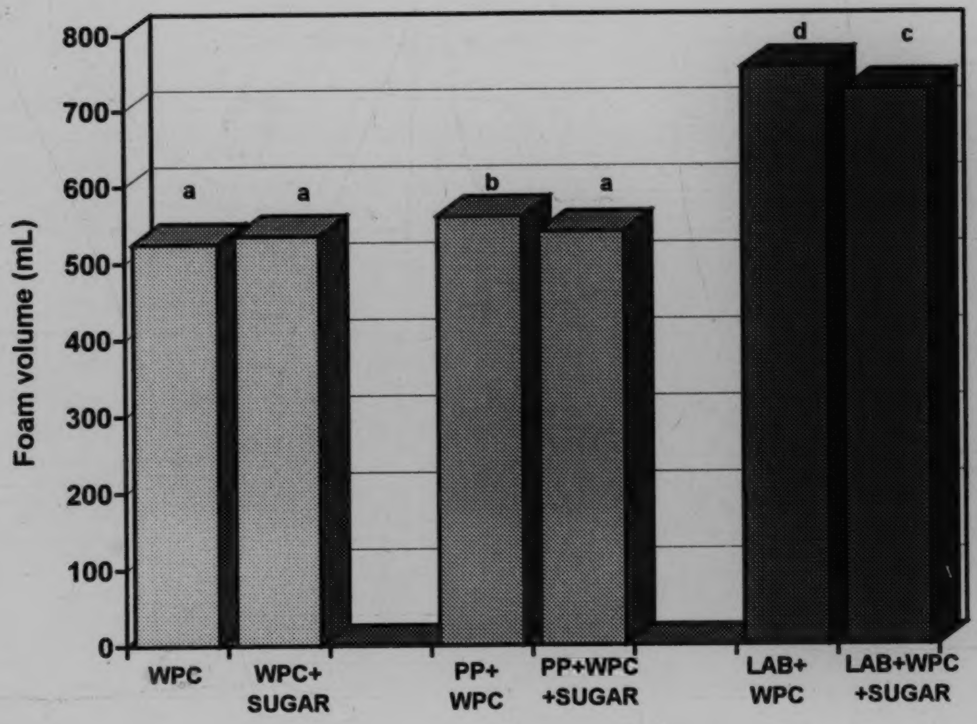

Figure 7.5. Volume of foams prepared with 5% WPC, 1% PP or LAB gum and 10% sugar. Bars with the same letter are not significantly different at $p>0.05$ level.

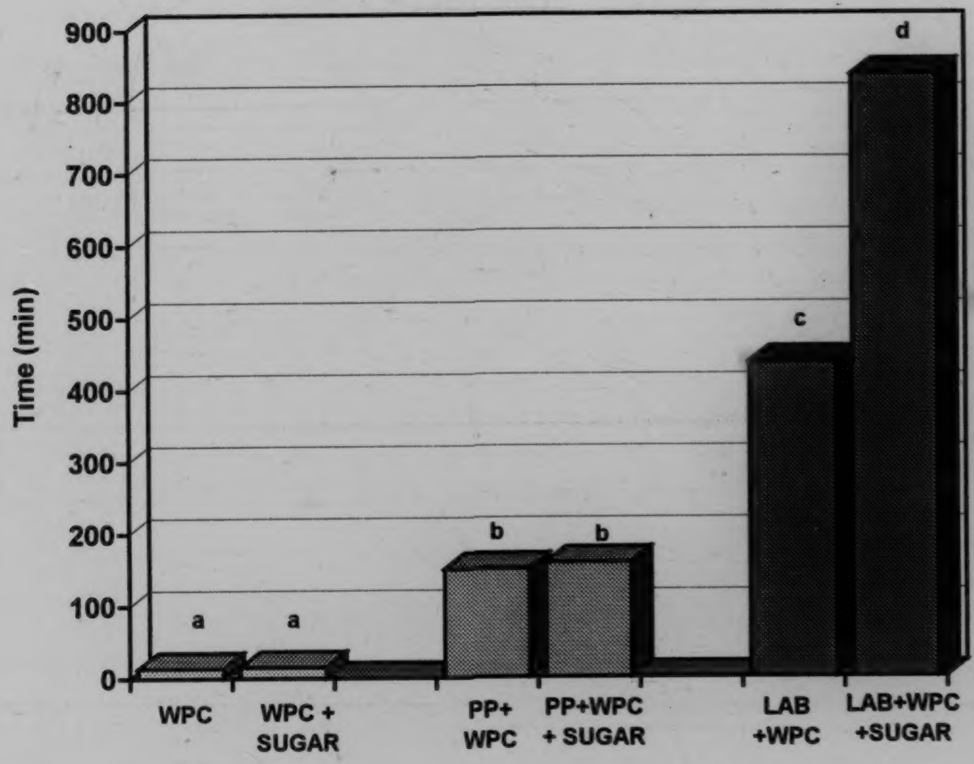

Figure 7.6. Time to 50% drainage of foams prepared with 5% WPC, 1% PP or LAB BBG gum and 10% sugar. Bars with the same letter are not significantly different at $p \leq 0.05$ level.

concentration were stable for only approximately 15 min (Fig. 7.6). Sugar addition to WPC did not extend drainage time significantly, but the addition of PP gum prolonged drainage time to approximately 2.5 h, while adding LAB gum promoted stability in excess of 7 h.

Sugar addition showed a tendency to suppress foam volume (Fig. 7.5) when BBG was present and reached a significant ($p \leq 0.05$) level in the case of LAB gum. This is possibly because of the viscosity increase from 24.9 to 27.6 mPa s, which decreased expansion. Viscosity of the LAB + WPC mix was so high that it was beyond the range of our viscometer. Subsequent addition of sugar resulted in a visibly thicker solution. Stability was not affected ($p > 0.05$) by the addition of 10% sugar except when it was combined with LAB gum. The depletion of free water by sugar hydration could limit the drainage of foams and improve stability.

It was not possible to prepare foams with either BBG gum alone at 1 or 2% concentration. The higher concentration (2.5%) of LV BBG used by Temelli (1997) was sufficient to form a foam, probably due to the creation of a network with a pronounced yield point. In addition, LV gums create a gel network much faster than MV and HV gums, which require more time (Burkus and Temelli 1999). The main mechanism of foam and emulsion stabilization by LV BBG gums seems to be the formation of a network, as confirmed by DSC and gelation tests. A similar network created by carrageenan in chocolate milk prevents settling of cocoa particles (Glicksman 1969). LV gums must be applied in sufficient concentration (>2%) for a network to form quickly, and thickening will also contribute to the stability. In that case, both mechanisms act simultaneously. On the other hand, MV and HV gums first act by increasing viscosity, while network formation needs time, up to several days, depending on the concentration and molecular weight. Longer molecular chains require longer setting time (Burkus and Temelli 1999).

7.4. CONCLUSIONS

BBG gums obtained at pilot plant and laboratory scale exhibited mild surface activity, probably due to the presence of impurities such as protein, which would not be expected to have a significant impact on the stability of foams and emulsions at the levels studied. BBG forms a reversible gel network, which starts melting in the range of 58-63°C and finishes (probably) at <70°C. Medium viscosity PP gum and high viscosity LAB gum alone at a level of 1 or 2% (w/w) did not form foams and did not stabilize emulsions when applied alone at 1% (w/w). When WPC was used as an emulsifier and foaming agent, BBG addition increased ($p \leq 0.05$) the stability of foams and emulsions. Sugar addition (10%) increased ($p \leq 0.05$) the stability of foams only when applied with HV gum. The main mechanism of foam and emulsion stabilization by BBG gum is due to increased viscosity. Yield stress caused by the formation of a β-glucan network may be an additional stabilizing factor and requires further investigation.

7.5. REFERENCES

Autio, K. 1988. Rheological properties of solutions of oat β-glucans. Pages 483-488 in: Gums and Stabilizers for the Food Industry 4. G. O. Phillips, P. A. Williams and D. J. Wedlock, eds., IRL: Washington, DC.

Autio, K., Myllymaki, O., and Malkki, Y. 1987. Flow properties of solutions of oat β-glucans. J. Food Sci. 52:1364-1366.

Bansema, C. 2000. Development of a Barley β-Glucan Beverage With and Without Whey Protein Isolate. M. Sc. Thesis. University of Alberta, Edmonton, AB.

Bhatty, R. S. 1995. Laboratory and pilot plant extraction and purification of β-glucans from hull-less barley and oat bran. J. Cereal Sci. 22:163-170.

Burkus, Z. 1996. Barley β-glucan: Extraction, Functional Properties and Interaction with Food Components. M. Sc. Thesis. University of Alberta, Edmonton, AB.

Burkus, Z., and Temelli, F. 1998. Effect of extraction conditions on yield, composition and viscosity stability of barley β-glucan gum. Cereal Chem. 75:805-809.

Burkus, Z., and Temelli, F. 1999. Gelation of barley β-glucan concentrate. J. Food Sci. 64:198-201.

Cao, Y., Dickinson, E., and Wedlock, D. J. 1990. Creaming and flocculation in emulsions containing polysaccharides. Food Hydrocol. 4:185-1959.

CRC Press, Inc. 1988. Handbook of Chemistry and Physics. 1st student edition. CRC Press: Boca Raton, FL.

Dawkins, N. L. and Nnanna, I. A. 1995. Studies on oat gum [(1→3, 1→4)-β-D-glucan]: composition, molecular weight estimation and rheological properties. Food Hydrocol. 9:1-7.

Dickinson, E., and Golding, M. 1997. Depletion flocculation of emulsions containing unadsorbed sodium caseinate. Food Hydrocol. 11:13-18.

Doublier, J. L., and Wood, P. J. 1995. Rheological properties of aqueous solutions of (1 →3) (1→4)-β-D-glucan from oats. Cereal Chem. 72:335-340.

Eastwood, M. 1987. Dietary fiber and the risk of cancer. Nutr. Rev. 45:193-198.

Gaonkar, A. G., 1989. Interfacial tensions of vegetable oil/water systems: effect of oil purification. J. Am. Oil Chem. Soc. 66:1090-1092.

Gaonkar, A. G. 1991. Surface and interfacial activities and emulsion characteristics of some food hydrocolloids. Food Hydrocol. 5:329-337.

Glicksman, M. 1969. Gum Technology in the Food Industry. Academic Press: New York.

Grimm, A., Kruger, E., and Burchard W. 1995. Solution properties of β-D-(1,3)(1,4)-glucan isolated from beer. Carbohyd. Polym. 27:205-214.

Gosain, K. 1996. Long Term Effects of Barley Bread Products on Metabolic Control of Non-Insulin Dependent Diabetes Mellitus. M. Sc. Thesis, University of Alberta, Edmonton, AB.

Kahlon, T. S., Chow, F. I., Knuckles, B. E., and Chiu, M. M. 1993. Cholesterol-lowering in hamsters of β-glucan-enriched barley fraction, dehulled whole barley, rice bran, and oat bran and their combinations. Cereal Chem. 70:435-440.

Li, D., Cheng, P., and Neumann, A. W. 1992. Contact angle measurement by Axisymmetric Drop Shape Analysis (ADSA). Adv. Coll. Interf. Sci. 39:347-382.

Linemann, A., and Kruger, E. 1997. Relationships between structure and characteristics of β-glucan in beer production. Part 1: Properties in dissolving β-glucan out of beer. Brauwelt Int. 1997 (IV):344-351.

Linemann, A., and Kruger, E. 1998. Relationships between structure and characteristics of β-glucan in beer production. Part 2: Changes in state of dissolution of β-glucan and formation of β-glucan gel during the brewing process. Brauwelt Int. 1998 (II):132-139.

McCleary, B. V., and Codd, R. 1991. Measurement of (1→3),(1→4)-β-D-glucan in barley and oats: a streamlined enzymic procedure. J. Sci. Food Agric. 55:303-312.

Morgan, K. R., and Ofman, D. J. 1998. Glucagel, a gelling β-glucan from barley. Cereal Chem. 75:879-881.

Newman, R. K., Klopfenstein, C. F., Newman, C. W., Guritno, N., and Hofer, P. 1992. Comparison of the cholesterol-lowering properties of whole barley, oat bran and wheat red dog in chicks and rats. Cereal Chem. 69:240-244.

Parker, A., Gunning, P. A., Ng, K., and Robins, M. M. 1995. How does xanthan stabilize salad dressing? Food Hydrocol. 9:334-332.

Phillips, L. G., Haque, Z., and Kinsella, J. E. 1987. A method for the measurement of foam formation and stability. J. Food Sci. 52:1074-1077.

Pick, M. 1994. Oat Bran Concentrate Bread Products: Long Term Effects on Diabetic Control. M. Sc. Thesis, University of Alberta, Edmonton, AB.

SAS Institute Inc. 1989. SAS/STAT User's Guide, Version 6, (4th ed. Vol. 2). SAS Institute: Cary, NC.

Temelli, F. 1997. Extraction and functional properties of barley β-glucan as affected by temperature and pH. J. Food Sci. 62:1194-1197, 1201.

Wendin, K., Aaby, K., Edris, A., Ellekjaer, M. R., Albin R., Bergenstahl, B., Johansson, L., Willers, E. P., and Solheim, R. 1997. Low-fat mayonnaise: influences of fat content, aroma compounds and thickeners. Food Hydrocol. 11:87-99.

Wood, P. J., Weisz, J., Fedec, P., and Burrows, V. D. 1989. Large-scale preparation and properties of oat fractions enriched in (1→3)(1→4)-β-D-glucan. Cereal Chem. 66: 97-103.

Wood, P.J. 1993. Physicochemical characteristics and physiological properties of oat (1→3),(1→4)-β-D-glucan. Pages 83-112 in: Oat Bran. P.J Wood, Am. Assoc. Cereal Chem. Inc.: St. Paul, MN.

8. CONCLUSIONS AND RECOMMENDATIONS

β-Glucan has been reported to have numerous health benefits. Such reports, together with the nutritional recommendation to increase fibre intake (Health and Welfare Canada 1990), have generated interest in isolating β-glucan from barley and oat with the ultimate goal of incorporating β-glucan gum into food products. In order to compete with other food hydrocolloids, barley β-glucan (BBG) must be stable, have high purity and be cost competitive. The viscosity type (i.e. high or low) of the extracted β-glucan varies depending on the barley cultivar and the extraction conditions, which may be dictated by the intended application of the β-glucan gum. As well, the behavior of BBG under different processing and environmental conditions, such as high shear, freezing and thawing (F-T), extreme pH and temperature, etc., must be known prior to large scale extraction or application. In addition, the physical and rheological properties of BBG, with the exception of flow behavior, have not been reported in the literature in great detail and, usually, properties of oat β-glucan are assumed for BBG. This thesis research attempted to fill some gaps in BBG knowledge and facilitate its applicability.

High viscosity (HV) BBG gum with purity ≥78% was extracted in the laboratory (LAB) at pH 9 and 55°C, and further stabilized using heat at approximately 90°C for 1 h at pH 9. Applying the same conditions during pilot plant (PP) extraction resulted in a lower yield (3.5% vs. 5.5% for LAB gum) of a higher purity (83.3%) and stable BBG gum, but with a low viscosity (LV) of 18 mPa s vs. 668 mPa s for 1% (w/w, as is) LAB gum solution at 12.9 s^{-1}. This large difference in viscosity is a consequence of a long holding time at elevated pH and temperature and, especially, high shear during extract purification and gum separation from ethanolic waste. The viscosity difference between LAB and PP gum was reported previously by Wood et al (1989). Experience gained after PP extraction led to some conclusions regarding the simplification and cost effectiveness of BBG extraction at a larger scale.

Simplification of an extraction process lowers the price of final products. It is necessary to keep extraction as simple as possible because scaling it up to an industrial level tends to make the process more complicated anyway, e.g. the need for 2-step centrifugation instead of 1-step to achieve the same separation since the g-force applicable in the laboratory cannot be attained with pilot plant equipment. Wood et al (1978) provided the basis for many of the more recent extraction studies. Using a water:flour ratio of 10:1, as proposed by Wood et al (1978), did not create any problems near neutral pH, but when the extraction pH was increased to ≥9, the level of impurities in the final product exceeded 30% (Burkus 1996). Protein and starch simply stayed suspended in the thick β-glucan extract. In that case, it is necessary either to use ultracentrifugation, or to dilute the extract more than the applied 12:1 water:flour ratio in this study, neither of which leads to simplification of the process but, rather, elevates the problem of impurities through decreased viscosity and lower suspending ability of the extract. At the pilot plant scale, proper equipment and/or timing (i.e. smaller batches) should be applied in order to keep the extraction process moving faster. That should also improve yield and viscosity by keeping the extract at high pH for the least amount of time possible.

In order to separate proteins, the Wood et al (1978) procedure requires pH adjustment to 4.5 and that has become a customary processing step. Based on the results of this laboratory, the same effect can be achieved with heat, which denatures and precipitates proteins. However, the problem is that because of the suspending ability of β-glucan, this coagulation is not readily visible. Evading the pH 4.5 precipitation step should also improve the purity of both the gum and the protein precipitate by decreasing the level of salt formation after neutralization of the extract. Heat must be applied in order to stabilize the β-glucan extract and gum (Burkus and Temelli 1998). Heated (boiled) gums would routinely have protein contents below 3%, while acid precipitated gums would contain >5% protein, even after extraction at neutral or lower pH, resulting in lower viscosity and poor suspending ability. Therefore, proper application of heat may help in the simplification of the extraction process. The effectiveness of heat as a means of gum stabilization and protein precipitation at pH<9 requires further experimental confirmation.

In terms of scale up, ethanol precipitation of β-glucan is by far the most problematic step. At the lab scale, ethanol can be handled without special restrictions, while at pilot plant or industrial scale the use of ethanol requires special considerations. Use and disposal of food-grade ethanol requires a special government permit, an explosion proof building and a spark-free equipment. Ethanol recovery and concentration through distillation, although not a step toward simplification, would surely increase cost effectiveness, but would require yet another government permit.

Morgan and Ofman (1998) made the first breakthrough using freezing as a mean to precipitate β-glucan. Because of a pending patent application, not all details were disclosed in their research note. While there is no doubt that freezing would be much simpler than ethanol precipitation, experiments in this research failed to reproduce the same result. Lacking detailed information, one can only speculate, e.g. a small amount of ethanol or salt could have been added to the β-glucan aqueous extract prior to freezing.

During freezing water crystallizes, while the concentration of salt or ethanol increases in the liquid water portion, reducing β-glucan solubility. β-Glucan molecules are pushed closer together and the concentration increases, which promotes gelling. The extraction process of Morgan and Ofman (1998) was driven by temperature-time variables in water without pH adjustment (25-55°C, 0.5-6 hr), which is basically allowing enzymatic hydrolysis to happen. β-Glucan gum obtained by Morgan and Ofman (1998) procedure was of LV or medium viscosity (MV) type. Such a gum would be expected to gel easier than HV β-glucan obtained in a different fashion without hydrolysis (Burkus and Temelli 1999).

Cost effectiveness is the major obstacle for wider application of β-glucan. Today, barley varieties with a very high β-glucan content, such as Prowashonupana (17.4% β-glucan) are available. Fractions of higher β-glucan content (20-30%) may be obtained by simple mechanical operations such as pin milling and air classification (Vasanthan and Bhatty 1995) or pearling (Zheng et al 2000). Aqueous extraction is not expensive if it can be done semicontinuously with synchronized capacities of equipment. As mentioned previously, ethanol recovery may be the main cost consideration. That would be dependent on plant capacity and obtaining a proper government permit. But even in that case, processing of barley solely for β-glucan and dumping all byproducts into feed may still not yield a competitively-priced hydrocolloid except for, possibly, the pharmaceutical and cosmetic industries. For wider food applications, besides cost, β-glucan has to provide a competitive advantage over other food hydrocolloids through its physiological and/or textural functionality. Therefore, utilization of other barley components should be evaluated through fractionation of barley into the following streams:

- soluble fiber rich in β-glucan,
- starch, 100% waxy or as desired for other industries (paper),
- protein-rich fraction for food, feed or cosmetics,
- fiber-rich fraction, mostly insoluble fiber, preferably for food applications, and
- lipids rich in vitamins and antioxidants.

Economical barley processing should contain at least the first two streams, β-glucan as the high-priced product and starch as the most abundant component of barley grain. The protein precipitate (Chapter 2) contains >50% protein and >20% lipid (dm basis) and represents an excellent starting material for economically feasible extraction of barley lipids. The whole area of barley protein functionality has not been investigated but may yield a few pleasant surprises.

The molecular weight (MW) of PP and LAB gums produced in this study was determined using intrinsic viscosity measurements and was found to be 198,000 and 598,000, respectively, using linear extrapolation from the MW standard curve. Determination of the critical concentration c* for HV LAB gum solutions follows well the equation $c^*[\eta]\approx 2.5$ determined by Doublier and Wood (1995) for HV oat β-glucan. Application of the same formula to the LV BBG gums is probably out of the question because of its time dependent aggregation-gelation behavior.

Very low viscosity Condor BBG gum (4-5 mPa s, 1% w/w) underwent quick network formation when prepared at a concentration of 5%, with elastic modulus, G', exceeding viscous modulus, G'', after 1.5 h. The more viscous PP gum at the same concentration needed approximately 6.2 h to set a network. Decreasing the concentration to 2.5% extended the network setting time for Condor gum by at least 9-fold, while 2.5%

PP gum solution did not set a continuous network during overnight testing. However, G' increased confirming the concentration and viscosity dependence of time-related molecular associations in BBG solutions. HV LAB gum did not show any tendency to gel overnight.

Fresh solutions of BBG were not thixotropic at concentrations ≤1%, regardless of viscosity. After extended holding of LV gums, thixotropy can be expected as a consequence of network destruction. Network formation in more concentrated BBG solutions was responsible for the significant yield stress. At lower concentrations, network formation was slower but still present. However, Condor gum, at a concentration of 1%, does not have enough β-glucan to fill the solution volume and gel syneresis and phase separation can be expected. HV LAB gum at a concentration of 1% and shear rates <30 s^{-1} is highly pseudoplastic but with a relatively high flow behavior index of >0.8 above 15°C. Such pseudoplastic behavior indicates the potential for HV LAB gum to be used as a fat replacer. Conversely, PP gum was Newtonian with a flow behavior index >0.99, even at 0.1°C.

The differences in the behavior of LAB and PP gums were further elaborated after their solutions were submitted to high shear, freeze-thaw (F-T) cycles and long term storage treatments (Chapter 5). Ultrasonication treatment was detrimental to the viscosity of both LAB and PP solutions, while shearing with a Polytron homogenizer reduced the viscosity of LAB solutions by 15-50% in the 0.25-0.70% concentration range. Surprisingly, PP gum solutions were resistant to Polytron treatment at concentrations ≤1%, but 1% PP solutions were less resistant to F-T settling induced after the first F-T cycle and underwent a visible phase separation after the second F-T cycle. Laboratory gum, due to its HV, was more resistant to F-T. Refrigerated storage without freezing over a 4 week period also exposed PP gum as more prone to aggregation through increases in viscosity and pseudoplasticity. Laboratory solutions (1%) also exhibited a viscosity increase of 7.5% following 4 weeks of refrigerated storage. Increased viscosity of both gums at refrigeration temperatures slowed down the aggregation of BBG molecules.

When 1% PP gum solutions were held at room temperature for 4 weeks at different pH levels, the increase in viscosity and pseudoplasticity was more extensive, to the extent that PP solutions at pH 9 underwent phase separation. The aggregation was quite pronounced regardless of solution pH. Network formation in 0.5% LAB solutions was not detectable, but at 1% concentration and at room temperature, a pronounced increase in viscosity may be expected.

Viscosity loss over a 4 week period was notable for LAB solution only in the pH 2 buffer. Sensitivity of LAB gum to extreme pH conditions was increased at 55°C, with a significant ($p \leq 0.05$) drop in viscosity in pH 2 and pH 3 buffer; the surprise was its stability in a pH 10 buffer. At 90°C, the increase in pH sensitivity of the LAB gum was more pronounced than for PP gum. Laboratory solutions were stable for 1 h at 90°C and pH 5-8 when compared with a LAB gum control in distilled water, while PP solutions were stable in the pH range 5-9, with some change at pH≤4. Consecutive heating for 4 x 1 h at 90°C also demonstrated that thermal degradation is happening regardless of environmental pH.

The different behavior of LV PP gum and HV LAB gum is probably the consequence of differences in the micellar structure of the β-glucan aqueous solution. There is no doubt that β-glucan forms micelles. These micelles differ in size depending on the MW of β-glucan. HV β-glucan forms a larger number of bigger and fluffier micelles with fewer chains associated into multimers. There is no single form of micelle, but probably numerous transient forms created through two modes of aggregation; crystalline bonding of cellulosic regions and hydrogen bonding of regularly shaped helical sequences (Fig. 1.1.). Micelles may be able to further associate into flocks by creating weak bonds between free tails protruding into solution. Since flocks are not held together by strong bonds, they disassociate when more energy is provided to the solution in the form of shear or increased temperature. Therefore, there is a shear-thinning effect, which reaches a plateau when disassociation of flocks is near complete. LV β-glucan creates micelles as well, but ones which are probably more compact without long tails, and with a higher degree of association, so that the level of interaction and flock formation is lower. This kind of micelle behaves more like small spheres and the result is Newtonian behavior of LV β-glucan solutions. These more compact micelles may be more resistant to extreme pH due to increased steric hindrance.

Besides differences in the micellar structure, there is also a probable difference in the structure of the glycosidic polymeric chain of LV and HV β-glucan. The (1→3) bond may be also more sensitive to high shear (Chapter 5. The combination of high pH and high shear (Chapter 2) could preferably affect the (1→3) bonds during pilot plant extraction. Cleavage of these bonds would make more terminal (1→4) sequences available for creating stable crystalline cellulosic regions between β-glucan micelles and/or molecules, and would promote network formation (Chapter 4). That, in combination with the lowered hindrance of the low viscosity (LV) environment, may explain the increased ability of LV BBG gum to form a network. Even though the Scanning Electron Microscopy images provided some evidence for network structure, more detailed structural analysis is necessary to confirm some of the above speculation.

Thus, the applicability of LV PP gum at a given concentration (1%) may be limited to food systems where it does not undergo shear, and reheating may melt any created structures (i.e. sauces). Gel formation may even enhance physical and oxidative stability. The only problem may be some tendency toward syneresis. On the other hand, in stressed samples (i.e. ketchup), applicability of LV gum is probably limited to about 3 weeks at 1% concentration and by other factors such as thermodynamic incompatibility with other food components (Tolstoguzov 1991), or depletion flocculation in emulsions (Dickinson 1988), may make it even worse. However, lowering concentration and/or temperature (viscosity increase) may prolong the shelf life of such products.

Application of HV LAB gum to stabilize foams and emulsions (Chapter 7) was successful when used in combination with whey protein concentrate as an emulsifier and foaming agent. The low viscosity of PP gum could not prevent the initial creaming of a 50% oil-in-water emulsion. After 21 days, it gelled without further phase separation. The combination of HV LAB gum to create a high initial viscosity and LV PP gum to provide network support and yield stress may be interesting for further research. Preparation of emulsions without using the Polytron, which cleaves HV β-glucan, would be beneficial for emulsion stability.

Although neither PP nor LAB gum could create a stable foam by itself at a concentration of 2%, foam created by Temelli (1997) using 2.5% Condor gum was stable ≥2 h. The probable explanation is that beating may have speeded up network formation in the 2.5% Condor solution (Chapter 4) by forced aggregation. Molecular repulsions were instantly overcame by the force of the beater. The created gel with embedded air further strengthened over time, becoming even more stable. The only problem with an increase in gel strength may be syneresis.

Improper handling of food samples enriched in β-glucan may be detrimental for their stability as well as the physiological functionality of β-glucan. Beer et al (1997) demonstrated that freezing of muffins enriched in β-glucan lowered the solubility of β-glucan and its potential health effects. Extended frozen storage of bread for a clinical study is a possible reason for the reduced effects of barley bread on glycemic response and the serum cholesterol level of subjects (Gosain 1996). Just mixing of barley flour with water and keeping it for a few minutes at room or slightly elevated temperature (approximately 35°C) would be detrimental to water holding capacity just because of β-glucan degradation. Additionally, thermodynamic incompatibility between barley β-glucan and endogenous barley proteins probably exists and may become enhanced with heating. Plus, there is a possible incompatibility with proteins of the food system β-glucan is incorporated into. Consequently, enzymatic hydrolysis and incompatibility with proteins could be the reasons for unsuccessful application of β-glucan rich barley flour in salad dressings (Bhatty 1997) and in sausages (Shan 1996).

Therefore, if a health claim similar to that approved by the U.S. Food and Drug Administration (FDA 1997) for certain types of oat products is also approved for BBG rich products in the future, then such approval should also contain either precautions to ensure β-glucan stability, or handling recommendations to prevent and/or slow down BBG degradation and insolubilization during food preparation and subsequent storage. This will ensure the efficacy of physiological functionality of β-glucan upon consumption of β-glucan rich products.

To conclude, BBG is a hydrocolloid with high potential for utilization as a stabilizer, suspending agent and fat replacer in food systems. Its behavior is molecular weight, concentration and time dependent, and controlling these three variables controls the physical stability of β-glucan and determines the successful outcome of its applications. Although HV BBG gum seems to be *a priori* the desirable type of β-glucan, the aggregation behavior of LV BBG can probably be turned into an advantage for certain food and non-food (cosmetics, pharmaceutical, etc.) applications, and incorporation of different combinations of HV-LV BBG gums may be full of surprises. Resolving the cost effectiveness of BBG extraction, which is expected in the near future, would clear the way for wider utilization of BBG and real value-added processing of barley. As stated previously in M. Sc. Thesis (Burkus 1996), the era of β-glucan is yet to come.

8.1 REFERENCES

Beer, M. U., Wood, P.J., Weisz, J. and Fillion, N. 1997. Effect of cooking and storage on the amount and molecular weight of (1→3)(1→4)-β-D-glucan extracted from oat products by in vitro digestion system. Cereal. Chem. 74:705-709.

Bhatty, R. S. 1997. Personal communication. University of Saskatchewan, Saskatoon, SK.

Burkus, Z. 1996. Barley β-glucan: Extraction, Functional Properties and Interaction with Food Components. M. Sc. Thesis. University of Alberta, Edmonton, AB.

Burkus, Z., and Temelli, F. 1998. Effect of extraction conditions on yield, composition and viscosity stability of barley β-glucan gum. Cereal Chem. 75:805-809.

Burkus, Z., and Temelli, F. 1999. Gelation of barley β-glucan concentrate. J. Food Sci. 64:198-201.

Dickinson, E. 1988. The role of hydrocolloids in stabilizing particulate dispersions and emulsions. Pages 249-264 in: Gums and Stabilizers in the Food Industry. G. O. Phillips, P. A. Williams and D. J. Wedlock, eds. IRL: Washington, DC.

Doublier, J. L., and Wood, P. J. 1995. Rheological properties of aqueous solutions of (1→3) (1→4)-β-D-glucan from oats (*Avena sativa* L.). Cereal Chem. 72:335-340.

FDA 1997. Food Labeling; Health Claims; Oats and Coronary Heart Disease; Final Rule Federal Register Doc. 97-1598, filed 1-22-97.

Gosain, K. 1996. Long Term Effects of Barley Bread Products on Metabolic Control of Non Insulin Dependent Diabetes Mellitus. M. Sc. Thesis, University of Alberta, Edmonton, AB.

Health and Welfare Canada. 1990. Nutrition Recommendations. The Report of the Scientific Review Committee.

Morgan, K. R., and Ofman, D. J. 1998. Glucagel, a gelling β-glucan from barley. Cereal Chem. 75:879-881.

Shan, P. 1996. Personal communication. University of Saskatchewan, Saskatoon, SK.

Temelli, F. 1997. Extraction and functional properties of barley β-glucan as affected by temperature and pH. J. Food Sci. 62:1194-1197, 1201.

Tolstoguzov, V. B. 1991. Functional properties of food proteins and role of protein-polysaccharide interaction. Food Hydrocol. 4:429-468.

Vasanthan, T. and Bhatty, R. S. 1995. Starch purification after pin milling and air classification of waxy, normal, and high-amylose barleys. Cereal Chem. 72:379-384.

Wood, P. J., Siddiqui, I. R., and Paton, D. 1978. Extraction of high viscosity gums from oats. Cereal Chem. 55:1038-1049.

Wood, P. J., Weisz, J., Fedec, P., and Burrows, V. D. 1989. Large-scale preparation and properties of oat fractions enriched in (1→3)(1→4)-β-D-glucan. Cereal Chem. 66: 97-103.

Zheng, G., Rossnagel, B. G., Tyler , R. T., and Bhatty, R. S. 2000. Distribution of β-glucan in the grain of hull-less barley. Cereal Chem. 77:140-144.